An Ecological, Morphological and Molecular Investigation of *Beddomeia* Species (Gastropoda: Hydrobiidae) in Tasmania

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Declaration

This thesis contains no material which has been accepted for the award of any other degree or diploma by the University or any other institution. To the best of my knowledge the thesis contains no material previously published or written by another person except where acknowledgement is made in the text.

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Abstract

Narrow-range endemic species are thought to have naturally small distributions, limited by size, mobility, dispersal capabilities, specific habitat requirements and biogeographical boundaries. Many narrow range taxa are poorly reserved and most threatened freshwater invertebrate taxa in Tasmania, including the majority of *Beddomeia* species, fall into this category. Management of such taxa is reliant on informed ecological data which is not currently available for many freshwater invertebrates. To address the need for more detailed information on one such group of aquatic invertebrates, this study obtained spatial and ecological data for a number of *Beddomeia* species and identified habitat variables that together explained the snail distributions. A combination of geology, catchment size, forest type, flow and disturbance were identified as significant explanatory variables of *Beddomeia* presence within a river catchment. The distribution of some *Beddomeia* species is greater than previously predicted (at the catchment level), the *Beddomeia* spp. investigated were shown to occupy a wider number of streams than previously thought, suggesting that this is likely to be observed in other *Beddomeia* species. Detailed population structure data supported the previously held belief that *Beddomeia* spp. are long-lived and have low fecundity.

Anthropogenic disturbance to streams, resulting from agricultural and forestry operations has previously been identified as a potential risk to aquatic invertebrates, particularly narrow-range endemics, but this has not, until now, been tested for *Beddomeia* species. The effects of cable-harvesting forestry disturbance on a high density *Beddomeia* sp. population were investigated. Population data indicated a recovery of population structure, if not abundance, within five years post-harvest, suggesting a higher level of tolerance to disturbance than had been previously anticipated.

Sympatric associations between Hydrobiidae are not uncommon and were observed for *Beddomeia* spp. in many of the streams investigated. Several morphotypes from each of the two major study catchments, initially determined by shell measurements, were taxonomically described using a combination of external and internal characters that indicate a high level of intraspecific shell variation occurs, and failed to support the number of morphotypes identified using shell characters alone. The molecular taxonomic resolution within the genus *Beddomeia* was also explored, but the monophyly of the genus remains unresolved owing to the disparity of topologies recovered.

Results obtained from these studies are used to review the management of *Beddomeia* and indicate that current measures are likely to be sufficient for headwater streams supporting the *Beddomeia* populations investigated, but a precautionary approach is required for other species for which there is limited information, and extra conservation measures may be necessary for those species proven to be restricted to a low number of locations.

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Beddomeia tasmanica, live under microscope. Scale 800 µm.

1 Introduction

1.1 Hydrobiidae - a diverse group with narrow-range endemics

In many parts of the world, freshwater molluscs are well known for their ability to develop species-rich local radiations. Such radiations are recognised within such disparate groups as the Unionoidae within the Bivalvia (e.g. Lydeard and Mayden 1995), and, among the gastropods, the Glacidorbidae (Ponder and Avern 2000) and Pachychilidae (e.g. Rintelen *et al.* 2007). The Hydrobiidae, in particular, are a very diverse group, with radiations identified in such diverse genera as *Sulawesidrobia* and *Keindahan* from Lake Poso, Sulawesi (Haase and Bouchet 2006), *Belgrandiella* in Austria (Haase 1996), within the subfamily Nymphophilinae in North America (e.g. Sada 2001, Liu and Hershler 2005) and *Jardinella* from artesian springs in western Queensland (Ponder and Clark 1990) to name a few. Particularly large radiations have been recorded in southern Australia, for example within *Austropyrgus* (Clark *et al.* 2003), and in Tasmania, in the genera *Austropyrgus, Phrantela* and *Beddomeia* (Ponder *et al.* 1993, Clark *et al.* 2003) (see Section 1.5).

As with other invertebrate species in groups displaying such radiations (e.g., Australian burrowing crayfish (Horwitz 1990, 1994, Doran and Richards 1996, Hansen and Richardson 2006), cichlid fishes in Mexico (Sage and Selander 1975), Tasmanian cave fauna (Eberhard *et al.* 1991) and Tasmanian geometrid moths (McQuillan 2004)) a large proportion of species within the Hydrobiidae appear to have restricted distributions, being known from only single locations, small streams or limited areas (Harvey 2002, Ponder and Colgan 2002). As a result, many such species are considered rare or threatened and are listed on legislation aimed to provide protection and to improve their conservation status (Taylor and Bryant 1997, Driscoll and Sattler 1999, New and Sands 2003).

Both intrinsic and extrinsic factors influence the naturally occurring ranges of species (Ponder and Colgan 2002). Many species with naturally small ranges are limited by size, mobility and dispersal capabilities, (intrinsic factors) or by biogeographical boundaries such as geological barriers, soil changes, vegetation cover (Harvey 2002, Worthington Wilmer *et al.* 2008, Gaston 2009). Although historical biogeographical barriers are often invoked to explain small species ranges, for species with limited dispersal capabilities contemporary habitat barriers may be sufficient to prevent migration of species between potentially suitable habitats, as revealed by the apparent limited dispersal of crayfish between

discontinuous patches of buttongrass moorlands (Horwitz 1988, Hansen and Richardson 2002), or between streams within a catchment (Liu *et al.* 2003). These can have the same isolating effects as the real lack of connectivity between disparate waterbodies, such as in lake systems in northern Wisconsin (Heino and Muotka 2006), or between cave systems in Tasmania (Eberhard *et al.* 1991). Altitudinal barriers, or low rainfall and evaporation rates, may also disrupt the continuity of species' ranges; such effects can be seen in alpine vegetation (Crowden 1999) and in burrowing crayfish (Hansen and Richardson 2002). Narrow range endemics are the result of one or more of these factors, but depending on the ecological requirements of species, narrow-range endemicity may not in itself be sufficient reason for conservation concern, particularly where species occur within protected areas. However, where such range-restricted species occupy habitats in areas subject to human activity, for example agriculture, dam construction on headwater tributaries, road building, forestry, residential or commercial development, potential loss of habitat has been recognised as presenting problems for their conservation (Ponder 1997b, Little 1999, Sands 1999, Ponder and Colgan 2002, Ponder and Walker 2003, McQuillan *et al.* 2009). The radiations of hydrobiid snails in Tasmania present a typical example of these sorts of problems.

1.2 Ecology and Biology of Hydrobiidae

Recently published research has questioned the phylogeny of Hydrobiidae, finding it is probably polyphyletic and that Australasian species fall within a distinct clade (Tateinae), within a broad concept of Hydrobiidae (Ponder *et al.* 2008). This suggests that Australasian „Hydrobiidae' species should now be called Tateidae (W. Ponder pers. comm. 2010). This taxonomic change was introduced late in the writing stage of this thesis, as a result, has not been fully integrated into the text. For convenience the terms hydrobiid and Hydrobiidae have been retained, but will be reviewed when research is published. The reader is asked to consider this fact when reading this thesis.

Little is known of the ecology and biology of hydrobiid snails, or closely related families, due in part to their small size and cryptic nature. Aspects of their life-histories and habitats have been studied worldwide; however, most of this research has been biogeographical in nature, describing and explaining species distributions in lentic (e.g. Haase and Bouchet 2006, Lysne *et al.* 2007), lotic, including artesian springs (e.g. Hershler 1999, Hershler and Liu 2004, Sada *et al.* 2005, Hershler *et al.* 2008, Sada 2008) and estuarine locations (e.g.

Tanaka and Maia 2006). Less effort has been applied to aspects of predation on hydrobiids, but Morse and Lenat's (2005) investigation of predation on *Somatogyrus virginicus* by caddis larvae (Trichoptera: Leptoceridae), and predation on introduced hydrobiids by Chinook salmon in western U.S.A. (Bersine *et al.* 2008) are two of the exceptions. While molluscs in general (bivalves, Planorbidae/Physidae and other gastropods) have been reported as contributing to the diet of platypus (Faragher *et al.* 1979, Bethga 2001, McLachlan-Troup *et al.* 2009, Olsson Herrin 2009), stonefly nymphs (Plecoptera), introduced trout and platypus have been identified as predators of hydrobiids in Tasmania, (S. Munks & K. Richards unpublished data; J. Gooderham pers. comm.). The physiological responses of hydrobiids to predators are also poorly known, excepting that shell morphology of *Elimia livescens* has been observed to alter in response to the presence of crayfish (Krist 2002). Other aspects of research on the ecology and biology of hydrobiids include the physiological effects of salinity and temperature on brackish water species (e.g. Hylleberg 1975, Herbst *et al.* 2008) and recording the invasion of the New Zealand species *Potamopyrgus antipodarum* in various countries (e.g. Ponder 1988, Loo *et al.* 2007a, Naser and Son 2009). Some life history data have been collected for hydrobiids, or closely related families, however, these more frequently relate to species from hot spring environments (e.g. Mladenka and Minshall 2001, Sada 2008) or lakes (Lysne *et al.* 2007) and only limited life-history data is available for most described species. In Australia, the main focus has been on mainland stream and mound spring species, (e.g. Ponder *et al.* 1989, Ponder and Clark 1990, Worthington Wilmer and Wilcox 2007); relatively few studies have been conducted in Tasmania, or on Tasmanian species, the main exceptions being Clark *et al.* (2003), Perez *et al.* (2005), Ponder *et al*. (1993) and Ponder *et al.* (2005) (See Section 1.5).

1.3 Morphology and molecular studies

Identification of hydrobiids, and closely related families, to species level is challenging. Morphological convergence within the family has meant that traits such as shell characteristics, which are said to be „plastic' due to the variability within a species, are often convergent (two or more species may be very similar) and consequently are not reliable indicators of species (Hershler and Ponder 1998). Due to the small average size of species (between 2.0-6.3 mm within the genus *Beddomeia*, for example), and the great similarity in shell characters, field identification below genus level is very difficult, and sometimes impossible. To distinguish between species, several studies of hydrobiids (e.g. Ponder *et al.*

1993, Ponder *et al.* 1994, Hershler and Ponder 1998, Clark *et al.* 2003) have utilised anatomical characteristics as well as shell morphology.

Morphological taxonomy has been the primary means of classifying species for centuries. More recently, however, allozyme electrophoresis and DNA-based techniques have also been employed to determine species identity and relationships, particularly between morphologically hard to distinguish species such as weevils (Langor and Sperling 1997) and crayfish (Hansen *et al.* 2001), and these techniques have become widely accepted tools in studies of the systematics of the Mollusca (e.g. Dayrat and Tillier 2002, Lydeard and Lindberg 2003, Fukuda and Ponder 2005, Colgan *et al.* 2007, Ponder and Lindberg 2008). DNA sequencing and allozyme electrophoresis techniques have been applied as tools to resolve relationships within the Mollusca at the order and family levels, for example, to examine relationships between the loliginid squids (Anderson 2000), the cerithioidean gastropods from North America (Holznagel and Lydeard 2000), and the Unionidae (Lydeard *et al.* 1996). In addition, they have been used to determine speciation within genera, such as in the spring-snail genus *Bythinella* (Bichain *et al.* 2007), the marine pearl oysters *Pinctada* (Colgan and Ponder 2002) and the terrestrial genus *Tropidophora* from Madagascar (Emberton 1995), and to investigate genetic variation between populations of freshwater snails (e.g. Ponder *et al.* 1994, Hershler and Liu 2004). In the Tasmanian context, one study, conducted by Perez *et al.*, (2005) used several Tasmanian species, including *Pseudotricula*, *Nanocochlea, Austropyrgus* and *Beddomeia* species as part of phylogenetic analyses of the subterranean genera in this study, the only reported sequencing of any *Beddomeia* species to date.

Results of mtDNA and allozyme analyses can also provide alternate hypotheses about species relationships; for example, molecular studies on some groups within the Hydrobiidae, previously described from morphological characteristics, have revealed a lack of correlation between the morphological and genetic groupings, although not for all traits (Ponder *et al.* 1994). Molecular phylogeny and biogeography of spring-associated hydrobiids in North America as well as stream living taxa in Australia have also confirmed that allopatric populations showed higher genetic variability than could be discerned through taxonomy (e.g. Ponder *et al.* 1994, Hershler and Liu 2004). Such differentiation is not specific to molluscs; for example, similar patterns have been revealed in freshwater crayfish, using both allozymes (Hansen *et al.* 2001) and mitochondrial DNA (Munasinghe *et al.* 2004).

1.4 Conservation management issues

The necessity to manage short-range endemic species arises when such species are listed under threatened species legislation, either locally or nationally, or when species are recognised internationally, such as by the International Union for the Conservation of Nature (IUCN Species Survival Commission 2008). A prerequisite for appropriate management of such species is a detailed knowledge of the species' ecology and habitat requirements. Threatened species management, however, remains poorly funded in Australia, with limited resources available for research on rare, vulnerable or endangered species. Although research conducted on closely related, or threatened species elsewhere may provide the basis for predictions on aspects of life-history of unstudied species, extrapolation of such information to threatened species management is risky and must be considered carefully, especially in the absence of a well resolved phylogeny.

Nomination for listing under threatened species legislation is often precautionary, coming before the required information is available to: a) verify the need for listing (Taylor and Bryant 1997) and b) to assist in development of appropriate management outcomes. The first step in threatened species management frequently involves field surveys to identify the range of a particular species. Some species, like the hydrobiids, however, are not easily identified by non-taxonomists and consequently their addition to conservation lists is frequently questioned. Internationally, many aquatic molluscs face similar problems of recognition and subsequent management; consequently such species are more at risk of extinction. For example, the freshwater Pleuroceridae from North America represented one of the most endangered groups of organisms on that continent (Holznagel and Lydeard 2000) while other species of hydrobiids restricted to warm springs are in danger of extinction through trampling by visitors to the sites (Sada *et al.* 2005). This situation is further emphasized by Strong *et al*. (2008) who reflect on the proportionally high percentages of freshwater gastropods (~ 20%) in the recorded extinctions of molluscs, even though freshwater species comprise less than 5% of the total gastropod fauna. Failure to recognise radiations within genera is only part of the problem; without a full understanding of species and their habitat requirements, conservation of species is less likely to be successful (Ponder 1995, 1997b, New and Sands 2003, New 2007).

Listing itself may inhibit the gathering of further knowledge about threatened species by denying collectors access, on the assumption that specimen collection is a significant threatening process, even in situations where this is untested (Butcher *et al.* 1994, Yen and

Butcher 1997). The original taxonomic description or limited distributional studies are frequently the only information available about threatened species, particularly invertebrates. The threatening processes that led to their listing are often extrapolated from other sources, and have often not been tested.

It has been suggested that forestry, agricultural and mining activities have a negative impact on native hydrobiids, since "survival of hydrobiid populations is largely dependent on the retention of riparian vegetation which shades and protects the stream" (Ponder *et al.* 1993, Ponder 1997b, Ponder and Walker 2003). The effects of such activities have not yet been quantified, but they are likely to range from the short to long term, and some may be permanent. Changes in water quality, such as increased turbidity, sedimentation (Gomi *et al.* 2005, Rashin *et al.* 2006) and salinity (Mitchell and Richards 1992, Metzeling 1993, Blinn and Bailey 2001), increased organic inputs, higher water temperature, changes in flow (Bren *et al.* 2006) and altered metabolic signatures (Clapcott 2007) may either improve or reduce habitat quality for various aquatic species, resulting in alteration of stream biodiversity (Growns and Davis 1994, Ryan *et al.* 2001, Suren 2005). Permanent or longer-term change to macroinvertebrate community composition is also thought to arise through loss of habitat caused by structural changes to channel morphology and loss of riparian vegetation (Mazeika *et al.* 2004, Davies *et al.* 2005a, Davies *et al.* 2005b, Rabeni *et al.* 2005). Alterations in allochthonous inputs may result from permanent removal of riparian vegetation, its replacement with inappropriate species such as *Salix* spp., or shorter-term removal through fire and windthrow following a forest harvest. Such changes have the capacity to alter the primary source of food and sheltering material available for a significant proportion of the macroinvertebrate community in streams (Read and Barmuta 1999, Rabeni *et al.* 2005).

Changes to forest overstorey composition, resulting from the conversion of native forest to plantation, also influence stream ecology. Such changes have been revealed in studies investigating the effects of *Pinus radiata* forestry on stream communities. As an example, in New Zealand Thompson (2002) reported that when established as a plantation, the effects of *P. radiata* on the stream community was to reduce stream productivity, both in terms of algae and macroinvertebrates, compared to native streams. The specific effects of plantation establishment on freshwater snails remain untested.

Alterations to water flow resulting from land clearance, whether for agricultural or forestry purposes, show patterns of short term flow increases followed by flow reduction as forests

regenerate or monoculture crops grow (Ruprecht and Schofield 1989, Vertessy 1999). In the forest context the level and extent of these patterns is thought to be dependent on a combination of forest type, level of precipitation, extent of clearance and subsequent land use (Putuhena and Cordery 2000, Sinclair Knight Merz. 2000, Lane and Mackay 2001, McIntosh and Laffan 2005). The impacts of such hydrological changes on hydrobiid snail populations in headwater streams remain unknown.

Water quality and channel morphology in forest streams are affected by features such as soil type and erodibility, forest type and rainfall (e.g. Davies *et al.* 2005b, McIntosh and Laffan 2005). The impact of forestry operations on stream water quality and channel morphology depends, amongst other things, on the method and size of harvest operation, retention and width of stream buffer, regeneration burn, soil type and erodibility (Ralph *et al.* 1994, Kreutzweiser and Capell 2001, Wells 2002, Lee *et al.* 2004, Thompson *et al.* 2009). The effectiveness of riparian buffers to limit impacts on water quality and other ecological components in production forests has been widely investigated and reviewed, both within Australia (e.g. Borg *et al.* 1988, Davies and Nelson 1994, Bunce *et al.* 2001) and internationally, including in New Zealand (e.g. Boothroyd *et al.* 2004), Canada (e.g. Fuchs *et al.* 2003, Vaidya *et al.* 2008), U.S.A. (e.g. Jones *et al.* 1999, Kiffney *et al.* 2003, Moore *et al.* 2005, Wilkerson *et al.* 2006, Moldenke and Ver Linden 2007) and Sweden (e.g. Hylander *et al.* 2002). The resulting consensus is that the presence of buffer strips is important to reduce alterations to stream water quality and channel integrity, although opinions differ as to minimum buffer width. One study undertaken by Kiffney *et al.* (2003) in British Columbia found that native coniferous forest buffers of >30 m were effective in limiting changes to periphyton and macroinvertebrate community composition. In a review of guidelines on riparian buffer widths from Canada and the United States, Lee *et al*. (2004) found that mean buffer widths (Canada and U.S.A. combined) differed, dependent on waterbody type and forest type, from 15.1 to 29.0 m, while Canada had wider buffers (33-58%) but in general most were sufficient to protect stream biota and habitats. In yet another study, undertaken in Western Australia, Borg *et al.* (1988) reported no significant effect on watercourses or water quality when stream buffers were reduced from 100 m to 50 m, whereas removing stream buffers in their entirety led to minor changes in stream channel profiles and algal blooms.

Retrospective studies conducted in Tasmania in areas harvested to leave no streamside buffers have shown some medium-term physical effects on headwater streams, including increased channel depths and changes to substrate particulate matter (an increase in coarseness), which may remain detectable up to 15 years after harvest (Bunce *et al.* 2001,

Davies *et al.* 2005b), whereas Clapcott (2007) observed continued change in metabolic signatures in forested headwater streams up to 15 years after logging. Davies and Nelson (1994), investigating the relationship between riparian buffer widths and the effects of logging on stream biota, concluded that the impacts of logging were significant only at buffer widths of < 30 m for streams with catchments greater than 2.5 km^2. Although these findings relate to *Eucalyptus* dominated forests, similar results have been reported for studies conducted in coniferous forests in British Columbia and Oregon (e.g. Kiffney *et al.* 2003, Danehy *et al.* 2007).

Headwater streams are generally thought to contain only a subset of the macroinvertebrate diversity recovered in larger streams, but importantly, they support a biodiversity found nowhere else in the catchment, including many narrow range endemics and genetically isolated species (Ponder 1994, Gomi *et al.* 2002, Heino *et al.* 2005, Meyer *et al.* 2007, Heino *et al.* 2008, Barmuta 2009). The ecology of small streams is thought to be more impacted by forestry activities than that of larger streams, as the level of riparian buffer strip protection afforded to smaller streams is lower than for streams with larger catchments (Forest Practices Board 2000a, McDermott *et al.* 2007). Despite the more substantial riparian buffer strips afforded to larger streams, their ecology is also influenced by upstream land clearing and forestry activities (MacDonald and Coe 2007), shifting the macroinvertebrate composition from that typifying slow-flowing, naturally variable, forested streams to intermediately disturbed communities (e.g. Davies *et al.* 2005a, Smith *et al.* 2009), and reducing the ability of the stream to support native fish populations (e.g. Pusey and Arthington 2003).

The approach to the management of forested streams both within Australia and internationally is inconsistent and remains in flux. In a review of the forest codes of practice within Australia (prior to 2000), McCormack (1994) found that the protection afforded to streams varied between States, ranging from 5 m on temporary or intermittent headwater streams in Victoria, to 200 m for fifth order streams in Western Australia, while between 10 m and up to 30 m were designated in Queensland. There is still considerable variation in protection given to permanent headwater streams generally flowing throughout the year. For example, 10 m machinery exclusion zones on each side of the stream are used in Tasmania, 20 m reserves in Victoria, 10 m protection strips in New South Wales and 30 m, with a minimum width of 20 m, on first to third order streams in Western Australia (McCormack 1994). A subsequent review of Tasmania's forest practices code has since been undertaken; leading to additional protection measures afforded to some headwater streams in high soil erodibility locations (Forest Practices Board 2000a, Wells 2002, McIntosh and Laffan 2005).

1.5 Hydrobiidae in Tasmania

Using anatomical traits to review speciation within one hydrobiid complex from south-eastern Australia, Ponder *et al.* (1993) identified 67 species and separated the *Beddomeia* complex into four distinct genera; three of these genera (*Beddomeia*, *Phrantela* and *Nanocochlea*) are endemic to Tasmania, while the fourth genus, *Victodrobia*, only occurs in Victoria. Another diverse genus, *Austropyrgus,* occurs across Tasmania and southeast Australia, with species endemic to each State (Clark *et al.* 2003). The current distributions of Tasmania's hydrobiid genera reflect a degree of separation, particularly between the apparently closely related genera *Beddomeia* and *Phrantela*, with a lesser distinction between *Nanocochlea* and the externally morphologically similar *Austropyrgus*. No geographical separation is observed between *Austropyrgus* and *Beddomeia*, and almost complete overlap exists between *Nanocochlea* and *Phrantela*. Since 1993, a further genus, *Pseudotricula*, has also been identified from caves in southern Tasmania and additional species of *Nanocochlea* have been described (Ponder *et al.* 2005). *Austropyrgus* has a state-wide distribution (Clark *et al.* 2003), whereas the other genera display more localised distributions, as shown in Figure 1.1.

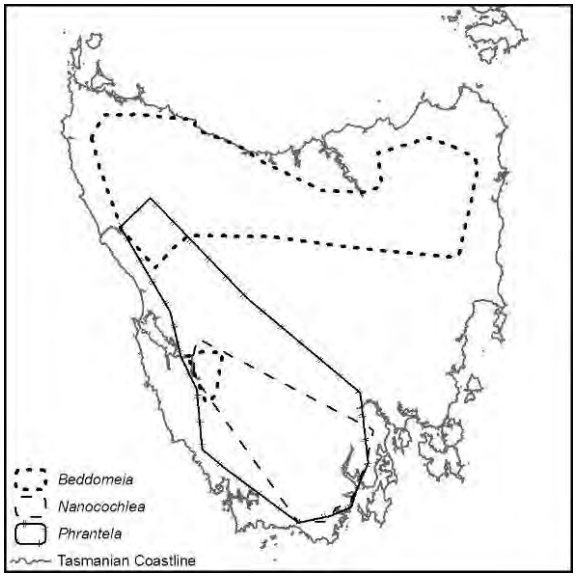


Figure 1.1 Distribution of Tasmanian hydrobiid genera *Phrantela*, *Beddomeia* and *Nanocochlea* based on information in Ponder *et al*. (1993) and Ponder *et al.* (2005).

The taxonomy of the Tasmanian genus *Beddomeia* has been under review for over a century, with taxonomic descriptions of various species published by Reeve (1857(-1858)), Tenison-Woods (1876), Johnston (1879), Petterd (1889) and Ponder *et al*. (1993). The seminal work

undertaken by Ponder *et al.* (1993) provides a detailed taxonomic review of the genus, describing 46 species, including four subspecies and a re-description of *Ampullaria tasmanica*, the type species of the genus *Beddomeia*. They suggest that up to 20 more species may be present; however, due to limited morphological differences or lack of adequate material these species were not described. More recently, two unpublished studies on one species in Tasmania, *Beddomeia launcestonensis*, have also been reported. The first, by Davies and Cook (2002), undertook a distributional study (status, management and critical habitats), while for his Honours thesis, Spiers (2003) investigated aspects of the species' feeding and phototactic responses, tolerance to flow and competition with other hydrobiids. These were the first studies to report on aspects of *Beddomeia* habitat preferences and tolerances in Tasmania.

Since all the *Beddomeia* species are dioecious, obligately aquatic and undergo direct development, their capacity for dispersal is limited; therefore, it is thought that most *Beddomeia* species have severely restricted ranges (Ponder *et al.* 1993, Ponder and Colgan 2002). However, no information is currently available on the characteristics of habitat utilised by most species, or on their population dynamics. To date, Tasmania's *Beddomeia* species, and indeed the family as a whole, are not well studied due in part to their size and their cryptic habits, living as they do in gravel, beneath rocks, amongst leaf packs or on logs in streams (Ponder *et al.* 1993, Ponder 1995). What little is known about their reproduction and behaviour suggests they have extremely limited dispersal capabilities (Ponder and Colgan 2002).

1.6 Current management

There are currently (March 2010) 183 rare, vulnerable or endangered animal species listed under the Tasmanian *Threatened Species Protection Act*, of which more than half (117) are invertebrates. The largest single group of listed invertebrates, containing 37 species, is from the genus *Beddomeia*; these species are also recognised as vulnerable by the IUCN (2008). Management of such species is governed by a series of parliamentary Acts (e.g. Tasmania's *Threatened Species Protection Act*, *1995*; *Nature Conservation Act, 2000*, *Environment Protection and Biodiversity Conservation Act, 1999*, strategies, policies and strategic plans (e.g. Tasmania's Threatened Species Strategy, DPIWE 2000, TSS 2006a, 2006b). Conservation measures are delivered through careful planning and application of rule-sets, e.g. the Forest Practices Code (Forest Practices Board 2000a, Munks and Taylor 2000), but

the effectiveness of this species management is currently hampered by insufficient knowledge of species' life history attributes, habitat preferences and responses to disturbance (Ponder 1997b, Ponder and Walker 2003, New 2007, Ilmonen 2008).

Within Tasmania's forestry estate, the current management prescriptions protecting streams are derived from soil and water research and are thus predominantly designed to ‚protect' soil and water values, with little direct reference to fauna and flora, although aquatic fauna was considered in the development of the rule-set and there is a recognition of the need for wider stream buffers in areas where listed species are known to occur (Forest Practices Board 2000a, 2001). In a comparison of international forest practice policies using Tasmania's Forest Practices Code as a standard for comparison, McDermott *et al.* (2007) identified significant differences between types and levels of protection provided to headwater and larger streams in different countries. The effectiveness of such management practices on threatened species, in particular aquatic invertebrates and fish species, remains unclear, although studies investigating the impacts of harvesting regimes, and the importance of riparian zones to invertebrate and fish conservation in the United States and Australia have identified changes to water quality, shading and allochthonous volume and composition as critical to fauna maintenance in streams (e.g. Davies and Nelson 1994, Growns and Davis 1994, Jones *et al.* 1999, Fuchs *et al.* 2003, Pusey and Arthington 2003).

It has been suggested that changes to streams brought about by land use practices, including agricultural and forestry activities, may in part be responsible for the absence of *Beddomeia* species in the lower reaches of some catchments (Ponder *et al.* 1993), however, this idea remains untested. While a few *Beddomeia* species occur in formally reserved areas, the majority occupy streams within Tasmania's production forests, and thus they are managed through reservation and agreed prescriptions developed in consultation with threatened species managers and delivered through the Forest Practices system (Forest Practices Board 2000a, b, Munks and Taylor 2000, Forest Practices Board 2001). This system relies in part on positive identification of species' presence; however, owing to the difficulty of identification of *Beddomeia* species, this is often difficult for land managers. An alternate management approach could be by ‚phenetic species-groups', i.e. groupings of species of *Beddomeia* based on similarity of morphological traits, as identified by Ponder *et al.* (1993), allowing these to be the unit of management rather than the individual species. This approach would only be effective if the habitat requirements of species within each species group are similar and a proportion of habitat for each species is maintained. While several ‚phenetic species-groups' by chance contain species that are generally found in close

proximity, many exceptions exist. The concept, which was introduced as an aid for identification only, was not meant to imply relationships (Ponder *et al.* 1993), and consequently is not likely to be useful for management purposes. As yet no research investigating habitat preferences of species within the phenetic species-groups has been conducted, nor has the molecular taxonomy of the genera been investigated to test the relationships of the species included in these groups.

Similar problems are being faced worldwide, wherever short-range endemics and the forest industry overlap. For instance, in Scandinavia and Europe considerable effort is now afforded to the protection of habitat for threatened saproxylic and log-dwelling invertebrates in managed forests (Martikainen 2001, Jonsell and Weslien 2003, Bouget *et al.* 2008), while in the Pacific North West of North America attention is focussing on riparian influences for the conservation stream biota including salamander, frogs and fish species (e.g. Olson *et al.* 2007). Limited funds are available to undertake individual autecological studies on the high number of forest-dependent threatened species in Tasmania, and given the cryptic nature, size and problems identifying species of hydrobiids, it is perhaps not surprising that so far only limited research has been completed, although being the largest single group (constituting > 25% of the threatened invertebrate species), it is concerning. Moreover, it is the forest industry and regulators themselves that support research of this type, to better assist in the management of these species. One such study is currently documenting the distribution and abundance of *Phrantela pupiformis* (Hydrobiidae: Mollusca) in a section of state forest adjacent to the World Heritage Area at Wedge, south of Maydena, Tasmania and will provide preliminary information on the impacts of one aspect of forestry on this species, (Davies *et al.* 2009), however, further research will need to be conducted on *Beddomeia*, a sister genus to *Phrantela*, to determine the effects on species of this genus.

1.7 Thesis aim and scope

The fundamental aim of this thesis is to provide information on the ecology and taxonomy of hydrobiids in Tasmania, with a view to improving their management. More specifically, it contributes to the systematics of *Beddomeia* species in Tasmania, examines range restrictions, specific habitat requirements, and evaluates the impact of forest harvesting on certain species of *Beddomeia*. Such information will facilitate the conservation management of this remarkable radiation of aquatic molluscs.

The first aim of this thesis is to characterise the habitat requirements of some *Beddomeia* species by examining their spatial and temporal distributions, variability and population structure. To address this aim a catchment-wide spatial and temporal survey was conducted across two catchments to identify, quantify and compare habitat selection of some *Beddomeia* species (Chapter 3). This study also investigated the relationship between habitat variables and abundance of *Beddomeia* and explored the hypothesis that different species of *Beddomeia* spp. have similar habitat requirements. To progress this aim, the development of an appropriate method for the survey is described in Chapter 2.

A second aim is to investigate to what extent anthropogenic disturbance, such as forestry operations, impact upon *Beddomeia* spp. and this was addressed through a retrospective survey investigating the effects of high level forestry disturbance on *Beddomeia* species and their potential recovery (Chapter 4).

The final aim is to determine whether sufficient genetic differentiation exists to warrant the current taxonomy of *Beddomeia* based on morphological characteristics. To address this aim the genetic differentiation of described species and the phylogenetic relationships within the genus were explored using mtDNA techniques to investigate the 16S and CO1 gene regions of some *Beddomeia* species (Chapter 6).

The information obtained will provide the basis for the suggested refinement of conservation management outcomes for listed species of *Beddomeia* (Chapter 7).

1.8 Overview of thesis

This thesis is divided into three sections. Section A comprises three chapters and reviews the development of an appropriate survey method for the ecological research, investigates habitat selection and distribution of *Beddomeia* across various spatial and temporal scales, and explores the impact of forest harvesting on some *Beddomeia* species. Section B comprises two chapters and explores the molecular taxonomy of some members of the genus *Beddomeia* and describes morphotypes discovered during this research, while Section C comprises a single chapter that presents a synthesis of research to date and discusses the issues associated with conservation management of narrow-range endemic invertebrate fauna.

1.9 Thesis format

Chapters 3 and 6 in this thesis are written in draft paper format; this will inevitably involve some repetition of material in the introduction and methods sections.

Section A Sampling methodology development and ecological studies

This section comprises three chapters and focuses on aspects of the ecology and distribution of *Beddomeia* spp. within two catchments in northern Tasmania. Habitat selection, distribution and population structure are examined at multiple spatial scales in exploration of the ecological parameters influencing *Beddomeia* spp. distribution.

Chapter 2 compares results of two potential sampling approaches to obtain spatial and temporal distribution and habitat data for hydrobiids, the more efficient method is then used to look for patterns of distribution (Chapter 3). Chapter 4 investigates the impacts of a specific method of forest harvesting (cable-harvesting) on snail abundance in northeast Tasmania. The aim of this section is to identify the most appropriate and efficient method for surveying streams to obtain ecological data for *Beddomeia* spp., to apply the methodology to investigate aspects of distribution and the potential impacts of forest harvesting on populations of *Beddomeia*.

Chapter 2

Development and comparison of two methods for use in ecological studies of hydrobiids

Sampling equipment, Groom River

2 Development and comparison of two methods for use in ecological studies of hydrobiids

2.1 Introduction

Sampling methods for freshwater molluscs reported in the literature generally fall into two categories depending upon the purpose of collection: either as a means of collecting specimens for systematic and phylogenetic investigations (e.g. Ponder *et al.* 1993, Ponder and Avern 2000, Goodacre 2002, Hyman *et al.* 2004, Haase and Bouchet 2006, Geiger *et al.* 2007), or to obtain data for comparisons of taxonomic diversity at given locations (e.g. Ponder and Clark 1990, Miller *et al.* 1999, Carlsson 2001). In either case, due to the nature of the research the sampling method provides only limited quantitative data.

Few studies have looked at the ecology of endemic freshwater molluscs, in particular, habitat preference (in the context of this study, defined as habitat on which snails are most abundant) and life histories. A recent study by Sada (2008), however, investigated microhabitat preferences and environmental factors influencing the structure of spring snail assemblages in North America. There have also been studies of the response of aquatic molluscs to environmental parameters, such as salinity gradients (e.g. Herbst *et al.* 2008) or disturbance through anthropogenic activities (e.g. Sada 2001, Sada *et al.* 2005).

Quantitative data for aquatic molluscs are more often obtained during general macroinvertebrate studies where snails are often considered by-catch. Survey methods used in such studies include sediment grab sampling, Surber sampling, sweep netting and kick netting, all of which may be used to collect snails. However, such methods either have issues of reproducibility, applicability for determining species habitat preferences, or scale, and as such they are unsuitable for the collection of data on molluscan life histories and population structures. While Surber or grab sampling may be used to target specific surface areas of streambed, the areas that can be sampled by such methods are insufficient to confirm the presence of hydrobiid snails in larger streams without multiple applications. This is due to the low density of snails in many larger streams, and the fact that they are also dependent on substrate type. Sweep netting requires aquatic vegetation to be useful, a feature not found in most small, forested, streams, while neither sweep or kick netting methods are able to effectively recover snails from rough surfaces of substrate. While many of these methods could be used to provide qualitative assessments of stream habitats which might provide

information on determining snail presence, in some instances, and may also provide valuable information for environmental review processes, with the exception of specific applications of Surber sampling targeting specific habitat types, none of the commonly used macroinvertebrate sampling methods are able to provide the quantitative data needed to explore the microhabitat preferences of aquatic snails, an important aim of this thesis.

The first part of this study evaluates the accuracy and precision of two methods commonly used to collect hydrobiid snails, the „visual assessment' (used by Davies and Cook 2002, Davies *et al.* 2009) and the „washing method' (e.g. Hershler 1999, Miller *et al.* 1999, Clark *et al.* 2003, Ponder *et al.* 2005), in determining snail presence and abundance on rocks in a stream known to contain *Beddomeia* species. Therefore study (1) was conducted to investigate the accuracy of the two methods as a means of measuring hydrobiid population densities, and to assess the methods in relation to sampling time, accuracy and applicability to larger ecological studies. The most accurate and efficient method was then tested and refined in the second part of the study (study 2), to establish a sample method for multiple habitat types, to gather data on *Beddomeia* spp. abundance along a stream gradient, and to use this information to design a sampling protocol for a catchment-wide study.

2.2 Methods

Study area

Studies were conducted in two tributaries of the catchment of Castra Rivulet, south of Devonport, in north-central Tasmania (Figure 2.1). The research site for study 1 was established on a headwater stream tributary of the Castra Rivulet, while the sites for study 2 were located on a headwater tributary of Deep Gully Creek. The tributaries were selected based on prior knowledge of hydrobiids (*Beddomeia* and *Austropyrgus* spp.) presence within the catchment.

2.2.1 Study 1: Comparison of sampling methods

Study sites

A single site of 20 m was established along a relatively undisturbed section of headwater stream, in mixed forest (wet *Eucalyptus obliqua* forest with rainforest understorey). Physical characteristics of the stream recorded at the site included channel width (average of 6 measurements), depth (average of 10 measurements) and stream gradient. Catchment size above the site was estimated from a 1:25000 mapsheet. For the purposes of this exercise,

data for *Austropyrgus* and *Beddomeia* species were combined, and two age classes (adult and juvenile), based on visual assessment of shell size, were recorded.

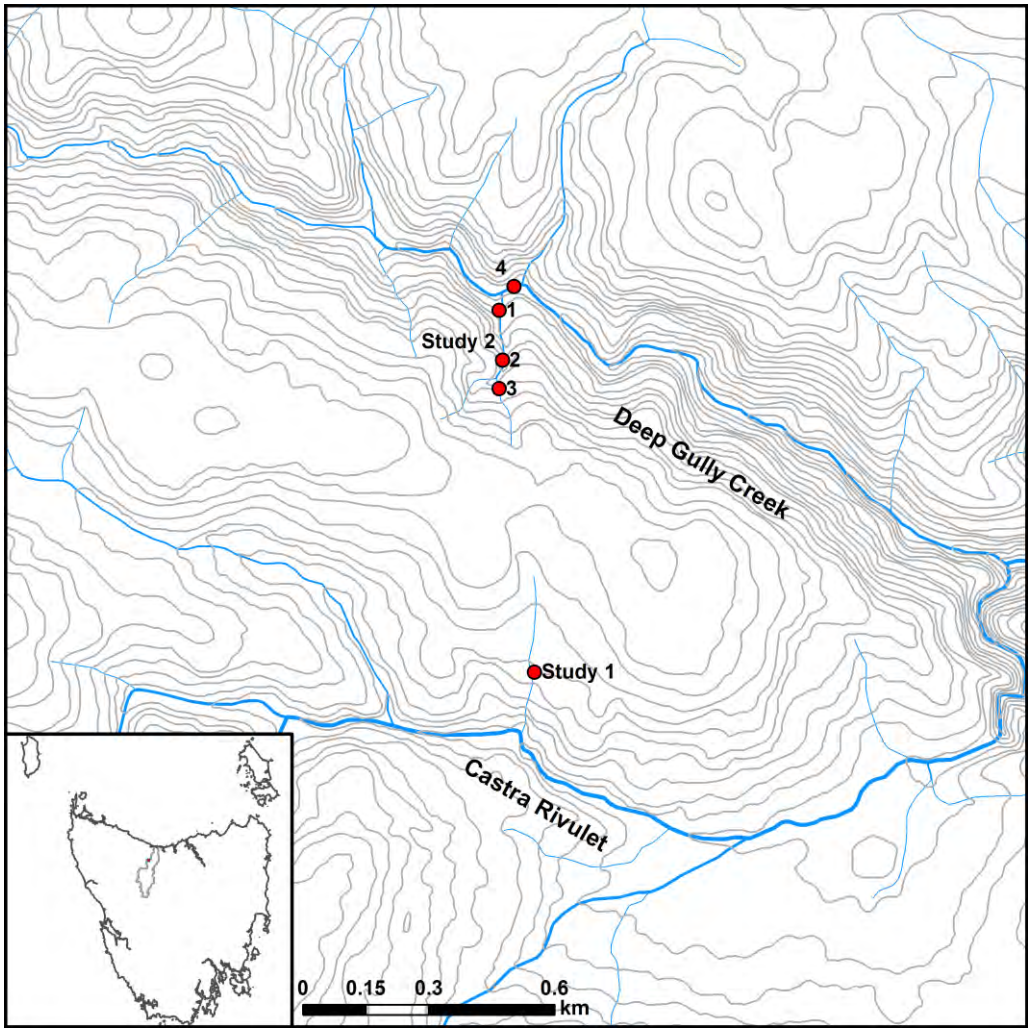


Figure 2.1. Location of study sites. Grid co-ordinates (in GDA) for Study 1 are 424873 5421355. Grid co-ordinates (in GDA) for Study 2 are: site 1 424589 5421355; site 2 424597 5422200; site 3 424589 5422319; and site 4 424624 5422376.

Snail sampling methods

Two methods, the visual assessment and washing method, were used to record snail numbers on rocks. Sampling time for the visual assessment component was restricted to a 3-hour period to establish an estimate of expected output using the visual assessment method (number of rocks sampled per time period). Following the visual assessment each rock was washed in a separate bucket of water using the washing method described below, and a sample obtained. No time limit was placed on obtaining samples using the washing method, but the time taken to process these samples was recorded.

Visual assessment (VA)

Previous application of this method involved searching all potential habitat types (emergent or submerged rocks, moss, immersed leaves and organic debris) for an hour, counting the visible snails present. No upper size limit of rock was defined; however, it is limited by rock weight (L. Cook, Freshwater Systems Pty, Ltd, pers. comm.). While site length is not pre-determined, sampling is restricted by time, and then the site length is recorded, from which snail density per 100 m of stream is calculated. No snails are retained using this method; hence it is limited to distinguishing between adults and juveniles only which limits the resolution of the data for detailed life history studies.

In this study, a subset of moveable submerged and semi-submerged rocks (of < 30 cm diameter) located throughout the site was individually surveyed during a three-hour period. Rocks were selected from different positions across the stream profile (edge or middle), and details of the rock features (size, geology, position of rock instream and level of submergence) were recorded. All molluscs and egg capsules seen on each rock were counted. To avoid counting errors, as snails were counted they were removed with tweezers and returned to the water downstream of the collection point following processing of each rock. A head torch was used because of the poor light conditions caused by dense canopy cover.

Ageing of snails was based on size; the large specimens of *Austropyrgus* and *Beddomeia* were counted as „adult' while smaller specimens were considered to be juveniles. *Austropyrgus* species at Castra are readily distinguishable from *Beddomeia,* possessing shells with narrow elongate spires compared with the broadly conic shape of *Beddomeia*, therefore there was no confusion distinguishing between adult and juveniles of each genus.

Washing method (WM)

The washing method, in which the defined habitat types (rocks in this instance) are washed in a bucket of water to obtain molluscan fauna, is a common technique used for recovering snails. The surface of each rock is agitated by hand in a bucket of water to remove all remaining snails. Each rock was categorized by size: boulders (> 25 cm diameter), cobbles (< 25 cm > 6 cm), or pebbles (< 6 cm) and recorded. Bucket contents were sieved following the processing of each rock, and the contents were transferred into individual vials; samples were sorted under a dissecting microscope in the laboratory to record the number of snails present.

2.2.2 Study 2: Testing and refining the 'washing' technique

Since no data on *Beddomeia* abundance or densities per rock were available to use as a basis, this study applied the washing method to investigate snail abundance at sites along a small headwater catchment to determine whether a linear trend in snail abundance could be detected: the null hypothesis being that no linear trend exists. To achieve this, a large site length (20 m) was established and all available rock and CPOM habitat within each site was sampled within a set time. Sampling was conducted over one day and rock sampling was conducted by one person to reduce operator variance. In addition, the method was applied to a second habitat type to provide confirmation of snail abundance trends and to explore predicted snail habitat preferences to determine the relevance to a catchment-wide survey. These data were then used to establish an appropriate stream length for study sites and to determine the amount of each habitat type to be sampled in order to minimize impacts on snail populations while allowing for changes in snail abundances to be detected.

Study sites

Three sites, each of 20 m in length, were established along the headwater stream, each separated by 150 m. Site 1 was immediately above the confluence with a fifth order stream (Strahler 1957), site 2 was located 150 m upstream and site 3 uppermost. Site 3 was located on the first order section of the tributary and sites 2 and 1 were situated downstream of the confluence on the second order stream: the second first order stream shown in Figure 2.1 was ephemeral and had minimal flow at the time of sampling. An additional site positioned in the fifth order stream below the sub-catchment was also surveyed (Site 4, Figure 2.1).

Snail sampling method

Two substrate types, rocks and coarse organic particulate material (CPOM), were selected for this study. Each substrate was sampled from within the site by one person for a

standardized period of 45 minutes. Rocks and CPOM were washed in separate buckets of water, the surface of the substrate being agitated by hand in the water to remove attached molluscs. At the end of the sampling time the contents of each bucket was sieved through a 300µm mesh and contents preserved in formalin or alcohol for later sorting. The washed rocks were categorized by size: boulders (diameter > 25 cm), cobbles (< 25 cm > 6 cm), or pebbles (< 6 cm) and counted. The CPOM was categorized by type: leaves, twigs, small branches, larger branches and the total volume of CPOM was estimated as a percentage of the tray.

Samples were examined under a dissecting microscope (Leica MZ75); snails were identified to genus and counted. Population age structure was not required for this part of the study and therefore was not determined.

2.2.3 Data analysis

Study 1: Comparison of sampling methods

ANOVAs were used to identify trends in the presence and abundance of snails, and potential habitat preferences. Snail abundances and life-history stages (adult or juvenile) recorded using each method were compared at the individual rock and total abundance per method levels. A *t*-test was conducted to compare the mean abundance of snails on basalt and siltstone rock types (sample size of 73 rocks (60 basalt and 13 siltstone) were conducted to determine the significance of substrate.

The percentage of snails recovered for during the visual assessment (VA) (=VA/(VA+WM*100)), and a comparison of the numbers of each size category (adults and juveniles) overlooked during the visual assessment were used as measures of accuracy of the method. Precision of the visual assessment method was determined by comparing snail numbers per rock before and after application of the washing method, and sampling times were evaluated by comparing level of output: the time allotted to sample using the visual assessment (three-hour period) to the time taken to sample and process the rock samples, both in the field and laboratory.

Projected estimates of field sampling time and sample processing time were used to evaluate the merits of each method. Estimates of the processing requirements for single and multiple habitat types for a catchment-wide study incorporating 54 sites were devised using sampling information obtained from both studies and are presented in the results Section 2.3.3.

Study 2: Testing and refining the 'washing' technique

Abundance data were standardized by volume of CPOM (snails per tray of CPOM) and surface area of rock (snails per cm^2 of rock) to estimate snail densities per site. Total abundance and standardized density were plotted for each snail genus by habitat type to identify potential linear trends in population density. Linear fits of total snail abundance versus site, and standardized snail density against site, for both *Beddomeia* and *Austropyrgus* spp. and ANOVAs were conducted on the data to determine significance of site as an explanatory factor of snail abundance. Non-parametric correlation coefficients (Spearman's Rho) were calculated for the total *Beddomeia* and separate habitat type datasets to test the ranking of sites.

Sampling efficiency

An assessment of the washing method to test operator sampling bias was undertaken, using four additional samples collected by three volunteers and one sample taken by the author for each habitat type. The following procedure was undertaken and repeated by each volunteer: samples of snails from CPOM and rocks were collected using the washing procedure outlined above. One full tray of CPOM was washed and the sample sieved and the contents preserved. Ten rocks of cobble dimensions (< 25 cm > 6 cm) were washed and the sample sieved and the contents preserved. An assessment of „sampling efficiency' was then conducted on the retained CPOM and rocks by more rigorously re-processing the material and taking a second group of samples. This was conducted by the author on each occasion. Again the samples were preserved and labeled. Samples were later examined under a dissecting microscope to recover all molluscs to obtain an estimate the proportion of snails missed.

2.3 Results

2.3.1 Study 1: Comparison of sampling methods

A total of 73 rocks were processed from the site during the three-hour visual assessment period. The field component of the washing method took thirty minutes; however, this was conducted in conjunction with the visual assessment fieldwork and also required a laboratory component, taking an additional three hours to process the 73 sample vials.

Snail counts

The presence of snails (*Beddomeia* and *Austropyrgus* species combined) was confirmed on 30 rocks using the visual assessment method, with no snails identified on the remaining 43 rocks. Microscopic examination of the samples collected using the washing method following the visual assessment method revealed additional snails on eleven of the 73 rocks, including three rocks on which the visual assessment method failed to record snail presence (thus the total number of rocks on which snails were recorded using both methods was 33). Sixty-seven snails were recorded using visual assessment, with a further 22 snails detected using the washing method (Table 2.1). Twenty-two adult and juvenile snails were recorded by the washing method, in even proportions, including three (one adult and two juvenile snails) on rocks for which the visual assessment method failed to confirm snail presence.

The numbers of each rock category sampled were recorded and are presented in Table 2.2. Snails were visible on rocks of each size category. They were identified on 41% of rocks sampled using the visual assessment method compared to 45% following the washing method. A total of 31 egg capsules were observed (visual assessment only); the majority of oviposition sites occurred on rocks classified as cobbles. Thirteen rocks upon which no snails were recorded supported egg capsules.

Table 2.1. Comparison of the numbers of snails collected by age category, for the Visual Assessment method (VA) and additional snails obtained by applying the Washing method (WM).

	VA	WM	Total snails
Adult	36	11	47
Juvenile	31	11	42
Total	67	22	89

Table 2.2. Comparison of snail and egg capsules recorded by rock category using Visual Assessment (VA) and Washing (WM) sampling methods.

Rock category	Total rocks sampled	Rocks with snails (VA)	Rocks with snails (WM)	Rocks with snails (WM only)	Total rocks with snails (VA + WM)	Rocks with snail & egg capsules	Rocks with egg capsules only	Total No. of egg capsules (VA)
Boulders	3	2	0	0	2	2	1	2
Cobbles	40	23	10	2	25	8	8	18
Pebbles	30	5	1	1	6	2	4	11

Rocks of two different geologies (basalt and siltstone) occurred in the stream, and snails were recorded on both geological types (60 basalt and 13 siltstone). The mean abundances of snails per rock (VA + WM data) were similar and a *t*-test was performed to test the hypothesis that snails are distributed randomly with respect to geology. The mean abundance of snails on siltstone was 1.6 (SD = 4.37) which was not significantly different from the mean abundance of 1.13 (SD = 1.89) on basalt ($t_{(71)} = 1.99$, $p = 0.52$). Interestingly however, the mean abundance of snails on the two geologies, using VA data only was significantly different (mean abundance of snails on siltstone was 0.923 (SD = 2.22) and the mean abundance of 0.916 (SD = 1.72) on basalt ($t_{(71)} = 1.99$, $p = 0.991$)).

2.3.2 Study 2: Testing and refining the 'washing' technique

Snails from both genera (*Beddomeia* and *Austropyrgus*) were present in each of the samples collected using the washing method taken at the three headwater stream sites, but no snails were recorded at the downstream site on the fifth order stream (Figure 2.1, Table 2.3).

Table 2.3. Total abundance of mollusca recorded at study sites.

Site	Substrate	*Beddomeia*	*Austropyrgus*
1	CPOM	20	258
1	Rocks	63	128
2	CPOM	89	311
2	Rocks	165	206
3	CPOM	235	285
3	Rocks	135	53
4	CPOM	0	0
4	Rocks	0	0

Snail abundance and species dominance varied significantly along the stream, with a linear trend of increased abundance observed in *Beddomeia* from the most downstream (site 1) to the uppermost site (site 3), while more *Austropyrgus* were recorded at the two downstream sites (Figure 2.2).

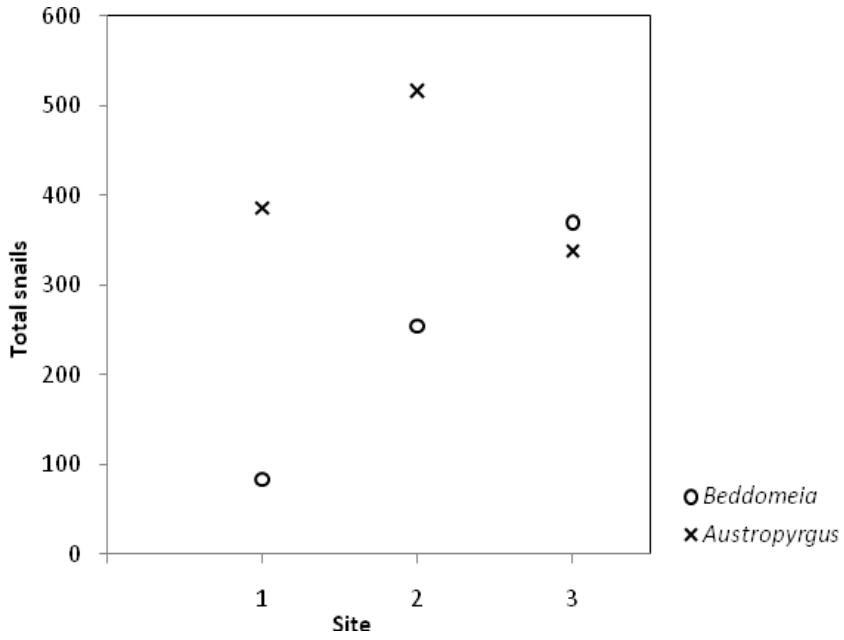


Figure 2.2. Total number of *Beddomeia* and *Austropyrgus* collected per site (rocks and CPOM data combined). Sites ordered by altitude; site 3 uppermost.

Spearman's Rho correlation coefficients were calculated against total snail abundance, total *Beddomeia* and total *Austropyrgus* abundance data to test the order of sites. The coefficient for site order using the total snails data was 0.50, (p>|Rho| = 0.6667), whereas for total *Beddomeia* was 1.00 (p>|Rho| = 0.00) and for *Austropyrgus* was -0.50, (p>|Rho| = 0.6667), indicating a clear ranking of sites for the *Beddomeia* abundance data only.

A comparison of the total numbers of *Beddomeia* and *Austropyrgus* recorded on each habitat type per site is presented in Figures 2.3 and 2.4, and suggests an increasing importance of CPOM as habitat along the stream in an upstream direction for *Beddomeia* whereas CPOM is always proportionally more significant compared with rock habitat for *Austropyrgus*.

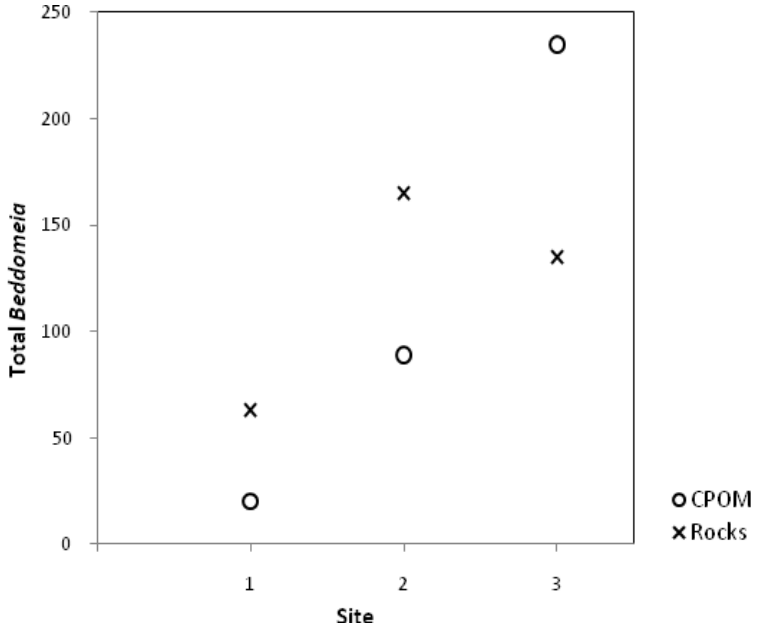


Figure 2.3. Total number of *Beddomeia* spp. recorded on rocks and CPOM at each site.

A Spearman's Rho correlation coefficient of 1.00 (p>|Rho| 0.0000) was obtained for total *Beddomeia* on CPOM, suggesting a strong ranking of sites, while a correlation coefficient of 0.5000 (p>|Rho| = 0.6667) was calculated for total *Beddomeia* on Rocks data, the latter indicating a poor ranking of sites. Such a result might be expected given the limited number of sites.

The Spearman's Rho correlation coefficients calculated for total *Austropyrgus* on CPOM and on Rocks were 0.50 and -0.50 (p>|Rho| 0.6667) respectively indicating no clear ranking of sites.

The results in the tables and figures above represent data gathered during a standardized time period, but with no accounting for changes in substrate volumes or amounts. Habitat availability varied between sites, with numbers of rocks washed ranging from 79 (site 3) to 120 (site 1). In addition, the amount of CPOM increased linearly up stream, with highest amounts at the stream headwater. The composition of the CPOM also changed, containing higher percentages of leaves at the upstream site. Standardizing the samples per unit area of habitat type (surface area of rocks and volume of CPOM) supports the trend identified by the raw data revealing increases in densities of *Beddomeia* (no. cm^{-2} of rock surface area and no. per volume (tray) of CPOM in an upstream direction (figures not shown for brevity).

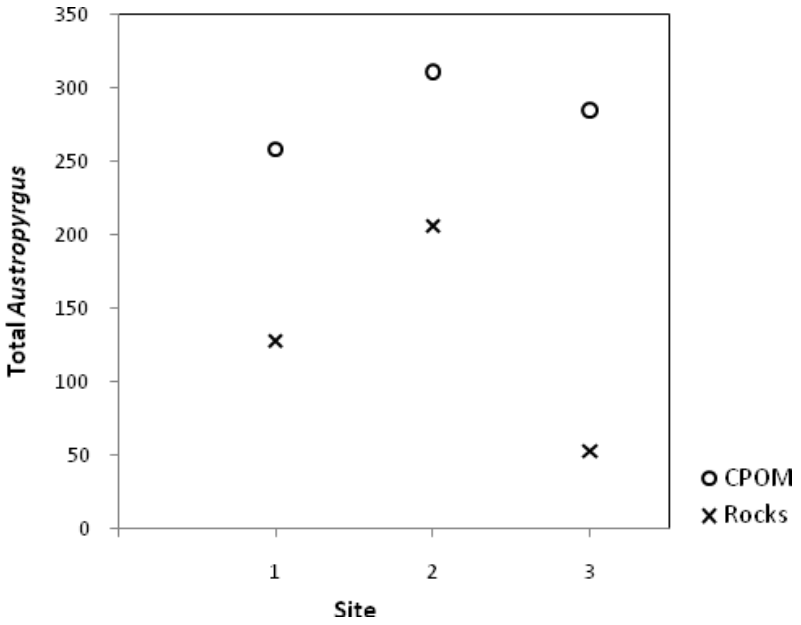


Figure 2.4. Total number of *Austropyrgus* recorded on rocks and CPOM at each site.

In contrast, trends in total abundances of *Austropyrgus* on both habitat types reveal elevated numbers at the lower two sites, with the highest numbers at site 2 and showed no linear correlation with site (figures not shown for brevity).

A bivariate plot of total *Beddomeia* against site reveals a strong correlation between *Beddomeia* abundance and position on stream ($R^2 = 0.9896$ and $p > F = 0.0651$), although the biological significance of this trend remains uncertain with only three data points (Figure 2.5). The corresponding ANOVA of this relationship is presented in the Appendix.

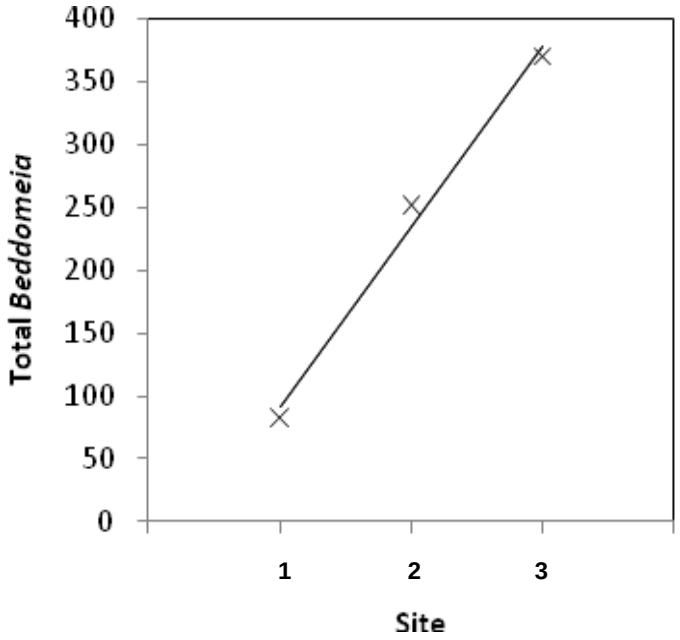


Figure 2.5. Plot of Total *Beddomeia* by site with line of best fit.

Similarly, a fit of total *Beddomeia* found on CPOM against site, although showing a similar pattern, had lower R^2 values (R^2= 0.955, Adj R^2= 0.9096) and the $p > F$ was not significant ($p > F = 0.136$). The fit of total *Beddomeia* on Rocks was also poor ($R^2 = 0.472$; Adj $R^2 = -0.057$; $p > F = 0.518$) due to a higher abundance of snails at site 2, and the relationship between standardized CPOM snail density (no. per tray) and site, and standardized rock densities (no. cm^{-2}) were not significant at $p = 0.01$. Bivariate fits of total and standardized *Austropyrgus* data against site showed very weak correlations, again due to the higher snail numbers at site 2.

The accuracy of the washing method differed depending on the operator (Table 2.4) and substrate type. Error margins for CPOM ranged from 0.47% to 9.76% for *Beddomeia* and between 1.02% and 9.09% for *Austropyrgus* (i.e. snails remaining in the CPOM after the first sample). Consistently fewer snails were missed by the author (Trial 5) and one other volunteer (Trial 3 and 4) notwithstanding higher *Beddomeia* abundances in the samples. Error margins associated with the rock substrate found the procedure to be more accurate; only one snail was missed on a sample of 10 rocks washed per volunteer, i.e. one snail on 50 rocks sampled.

Table 2.4. Results of sampling efficiency tests conducted on CPOM samples taken

Trial	Sample 1		Sample 2		Error (%)	
	Beddomeia	***Austropyrgus***	***Beddomeia***	***Austropyrgus***	***Beddomeia***	***Austropyrgus***
1	41	187	4	17	9.76	9.09
2	63	194	4	13	6.35	6.70
3	196	78	1	1	0.51	1.28
4	205	167	1	2	0.49	1.20
5	212	98	1	1	0.47	1.02

2.3.3 Sample processing

Processing times for in-field and laboratory sampling, for single and multiple habitat types, were estimated for a catchment study with 54 sites incorporating sampling information obtained from both studies (Tables 2.5 and 2.6). The two sampling methods have different in-field and laboratory sampling processing requirements. The visual assessment method has a long field-time component per habitat type, and if three habitat types are to be sampled, it is predicted that only a single site would be completed per day; however, this method uses no laboratory time. In comparison, the washing method has a relatively short field component, therefore allowing sampling of up to five sites a day, but has a lengthy laboratory component. (See discussion for details of Substrate sampling).

Table 2.5. Estimates of predicted field sampling times using Visual Assessment (VA) and Washing Method (WM) for multiple habitat types (rocks, CPOM and gravel/mud substrate).

Habitat sampled	Sites per day		Total days in field per sampling season	
	VA	**WM**	**VA**	**WM**
Rocks	2	5	28	11
Rocks + CPOM	1	4	54	13
Rocks + CPOM + Substrate	**1**	4	54	13

Table 2.6. Comparison of Visual Assessment (VA) and Washing Method (WM) sample collection and processing times for multiple habitat types (rocks, CPOM and gravel/mud substrate).

Habitat sampled	Time per site (hours)			
	VA (field)*	WM (field)	WM (lab)	WM (total)
Rocks	3	0.5	3	3.5
Rocks + CPOM	6	1	6	7
Rocks + CPOM + Substrate	7	1.2	7	8.2

* predicted for alternate habitat types CPOM and Substrate.

2.4 Discussion

2.4.1 Study 1: Comparison of sampling methods

The visual assessment method and the washing method are both useful in detecting the presence of hydrobiids in a stream. However, the results of this study indicate that the visual assessment method is not as accurate or efficient as the washing method when measuring abundance of snails.

Visual assessment was found to be less precise when determining the presence of snails on individual rocks. The presence of molluscs on some rocks was not detected, while half of the snails overlooked were adult specimens, which should have been detected. The washing method was more effective in recovering snails, both in terms of sampling time and the ability to collect without the need to establish the presence of snails prior to sampling, a benefit in low-light conditions.

The visual assessment method also underestimates the total number of snails, at all scales. Using this method, only 75 % of the total recorded snails in the study were detected leading to incorrect results for some individual rocks. More snails were missed on medium-sized rocks (cobbles), but inaccuracies in recognizing snail presence were independent of geology. Based on these findings, it is possible that the visual assessment method may fail to determine snail presence at all at sites with low snail population densities. An advantage of the washing method is that it can rapidly collect data from a site or multiple sites on a stream, while not being influenced by rock colour or size, or light conditions.

One benefit of the visual assessment method is that it is marginally more time efficient in sample processing time for a single habitat type at one site, and projected estimates indicate that the visual assessment remains more time-effective overall for obtaining data from one habitat. However, visual assessment requires a minimum of 2 ½ times the amount of field time for single-habitat sampling and there are limits on the type and quality of data that can be obtained using this method. The number of rocks assessed over a three-hour period was deemed to be low for the effort extended, and the level of population data obtained is minimal, since it is limited to adult and juveniles only. Less field time would have to be allocated to each site in order to sample multiple sites in a catchment-wide survey using the visual assessment method; however, this would impact upon the numbers of rocks feasibly sampled, limiting the usefulness of the study. Although the average laboratory sample-processing time for the washing method is longer than the time taken to complete the field-based visual assessment method, the data obtained using the washing method can offer insights on population structure and the co-occurrence of species.

As indicated, the quantity and quality of information obtained differs for each sampling method. One particular unique advantage of the visual assessment method is that it can detect the presence of *Beddomeia* egg capsules. Visual assessment can thus be used to evaluate hypotheses about preferred breeding positions across stream profiles. In this instance, an analysis of the position of rocks within the stream (edge, midstream) containing egg capsules failed to identify a preferred oviposition site, suggesting that flow in the headwater stream does not impede snail movement or egg laying. Similar numbers of capsules were recorded on rocks at the stream edge and mid-stream, and also on one rock sampled from on top of a pack of coarse particulate organic matter (CPOM), the likely result of substrate movement following higher than normal flows. Such information is important to our understanding of *Beddomeia* spp. behaviour and thus in this case visual assessment has a definite advantage over the washing method.

However, such advantages of the visual assessment method are outweighed by the serious time constraints on any large-scale surveys. The visual assessment method becomes a less attractive option at larger sampling scales, such as those required for the catchment-wide survey for the principle study in this thesis, particularly where multiple habitat types are incorporated into the study design. To realistically conduct a broad catchment-wide survey, application of the visual assessment method would need to be restricted to counting snails on a single habitat type in a given time frame and would restrict the amount of data able to be gathered. The advantage of the washing method over visual assessment here is that snails are

less likely to be overlooked and more habitat types can be sampled in a shorter period in the field. The washing method can readily be used to survey large volumes of material, taking less time to collect greater numbers of snails (in samples) and, after sample processing, it provides accurate data about population structure and allows the detection of different morphotypes, subspecies or multiple *Beddomeia* species. It can also be used to obtain by-catch data that may assist in explaining anomalies in the snail data.

This study only assessed the accuracy of the visual assessment method in detecting the presence of snails on rock habitat. However, the visual assessment method is also expected to be inaccurate when counting snails on allochthonous material due to the combination of increased surface area of this habitat type, its detailed surface structure (crevices) and the amount of material to be assessed. Visual assessment is also problematic where it is necessary to distinguish subtle differences in shell shape in the field. Therefore, even though improvements might be made by increasing the search time used in the visual assessment method (for rocky substrate), visual assessment is not considered the best method for this purpose.

One final factor worthy of consideration when selecting an appropriate method is the impact of the technique on the target species and by-catch, particularly when dealing with threatened species. On this criterion, the visual assessment methodology would undoubtedly be preferable. However, to obtain meaningful data, consideration needs to also be given to the error margins; an error factor of almost 25% does not allow great confidence in the results, particularly for sites with low snail abundances. In addition, the type of data which would be obtained, i.e. presence/absence and limited life-history data (adult vs juvenile) compared with more accurate population abundances, population structure, detection of morphotypes and the potential to collect associated macroinvertebrate community data, strongly suggests that the washing method is preferable for ecological studies.

2.4.2 Study 2: Testing and refining the 'washing' technique

Data collected using the washing method indicated that *Beddomeia* spp. appear to show a preference for headwaters of the streams (catchment < 1 ha). Increased number of snails present on rocks at the headwater site may be explained by the reduced flow allowing more surface area of rock to be utilized. However, the data also showed a decrease in the number of rocks present in the upper catchment. An alternate explanation is that the increased number of snails per rock reflects a behavioural change where higher densities are „tolerated'

due to the reduced availability of habitat. However, the same pattern of increase was observed in the total abundance and densities of *Beddomeia* spp. on CPOM, of which there was no shortage. At first glance, this might be explained by an increase in the amount of this habitat type available at upstream sites (more leaves, reflecting less stream power available to wash debris downstream) rather than an increase in population; however, even after standardizing the data (snails per volume of CPOM), there remains a significant increase in snail abundance per unit habitat.

Trends in the data for *Austropyrgus* spp. are less easy to interpret, with an increase in both the total and the standardized numbers of *Austropyrgus* spp. at the middle site (site 2) and no distinct linear trends in snail abundance, although care is needed proposing trends in data from such a limited number of sites, for both *Beddomeia* and *Austropyrgus*. It is clear, however, that the *Austropyrgus* species (possibly more than one) have a perceived preference for CPOM habitat over rocks. The increased number of *Austropyrgus* per rock at site 2 can be accounted for by the fact that snails in this genus utilize all surfaces of substrate (upper as well as beneath), whereas *Beddomeia* were only recorded on under-surfaces of rocks, on rock-substrate interfaces (where rocks touch soil or CPOM packs) or between layered CPOM. Further, at the uppermost site (site 3) there may be periodic desiccation of the upper rock surface in drier periods; however, this does not fully explain the differences between sites 1 and 2. The decline in available rock habitat, a possible preference for woody debris over rocks (when available), or reduced flows may all contribute to a partial explanation of the observed reduction in the *Austropyrgus* abundance at the headwater site.

No *Beddomeia* or *Austropyrgus* spp. were recorded from the site on the fifth order stream, suggesting that the hydrobiids in this catchment may be headwater stream specialists, a hypothesis supported by the high population densities in the smaller stream, particularly at the headwaters for *Beddomeia*. This hypothesis will be further explored in catchment-wide spatial study (Chapter 3).

One benefit of the washing method is its easy application to additional substrate types such as CPOM; however, there remained the question of its accuracy and precision for substrates other than rocks. Based on the results of the sampling efficiency testing, it can be concluded that washing of CPOM is the process most likely to have large error margins and that this is partly dependent on the individual conducting the sampling. Although these errors cannot be completely eliminated, they may be somewhat reduced by refining the method to include a combination of the employment of a standard method which is limited in time, places

restrictions on site length and specifies the volume of habitat material sampled (limited by volume of CPOM and number and size categories of rocks) and the use of trained field staff (to minimize observer error or bias).

2.4.3 Refining the methodology

The baseline data of snail abundance collected in this study has provided the following information that can be used to refine the washing method and sampling protocol for use in future studies:

- **Densities were high at all sites, thus the size of sampling sites can be reduced.** Three sites per stream, separated by a minimum of 100 m are probably sufficient. The total length of each site should be 20 m, incorporating four, 5 m sub-sites, each to be sampled once. This would reduce potential impacts on the species, but still be capable of detecting snail presence and offering meaningful data on population structure.

- **A range sizes were observed, suggesting that data on population structure can be collected.**
  Snails may be categorized into one of six size classes and counted, and any morphotypes recognised; this would assist in gathering life-history attributes of *Beddomeia* spp.

- **The proportions of different habitats varies between sites, thus sampling should be standardized.**
  To lessen the effect of variability between sites affecting the results, the method should aim to limit the volumes and amounts of habitat sampled at each sub-site. A maximum of one full tray (5 x 26 x 36 cm) of CPOM should be sufficient at each sub-site. The percentage and composition of the tray contents should be estimated; and sites where additional CPOM remains in-stream recorded. Proportions of CPOM should be representative of the sub-site, particularly where greater than maximum collectable CPOM volumes occur. Rocks sampled should include representatives of all three rock sizes, but be restricted to a total of 2 boulders, 25 cobbles and 25 pebbles; the number of rock types sampled being based on the average proportions observed over the 20 m sites in this study. In addition, sampling time should be restricted to 30 minutes per habitat type (rocks and CPOM).

- **Snails were observed on finer stream bed sediments, thus these habitats should be sampled.**
  A third habitat type of potential interest was also identified during this study. Some snails were observed on fine stream sediment, although it was impossible to ascertain numbers. Therefore, this habitat, the streambed substrate (gravel, mud, sand) should be included in any catchment-wide study of habitat use.

- **A means of estimating the surface area of substrates is required.**
  To further refine the method a means of determining the surface area of substrate washed was recognized as prerequisite to allow for the determination of a more accurate and precise calculation of snail density per area.

2.4.4 Sampling Protocol

The main aim of this thesis was to obtain information on the ecology of *Beddomeia*. To achieve this, it was proposed that a spatial and temporal investigation of *Beddomeia* spp. across two large catchments be undertaken. Employing the method above, it was proposed that the sampling protocol for this study include the following:

- Eighteen streams in each catchment would be surveyed and include streams of a range of stream orders (1 – 6, where possible).

- For consistency, streams were to be labelled using the catchment prefix Ca (for Castra) and GC (for Groom River at Goulds Country) and numbered from 1 to 18 in a clockwise rotation numbering system from the western-most site in each catchment (e.g. Ca1, GC3).

- Three study sites should be located on each stream.

- Sites would be numbered from the downstream site moving upstream, i.e. Site 1, stream 1 located approximately 20 m upstream of the confluence with another stream. Sites 2 and 3 spaced at 100 m intervals from the end of the downstream site.

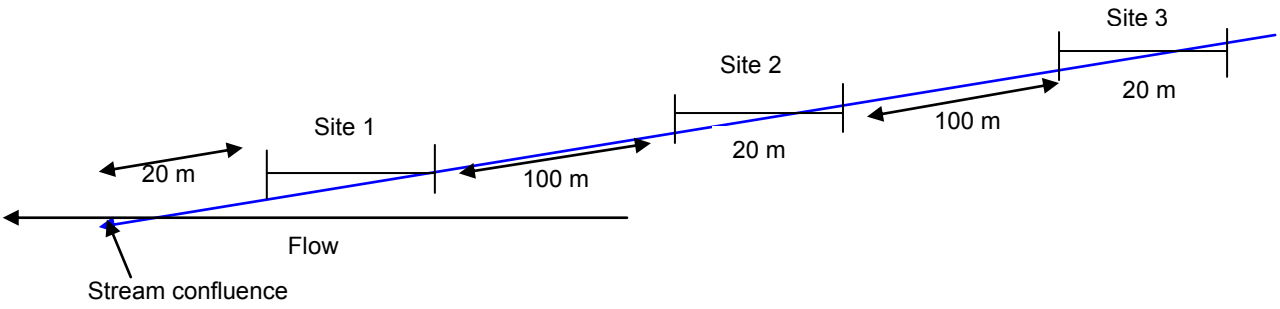


Figure 2.6. Illustration of site spacing and location

- Each site would be divided into four equal subsections of 5 m and labelled as sub-sites A, B, C, D, in an upstream direction, sub-site „A' being further most downstream.

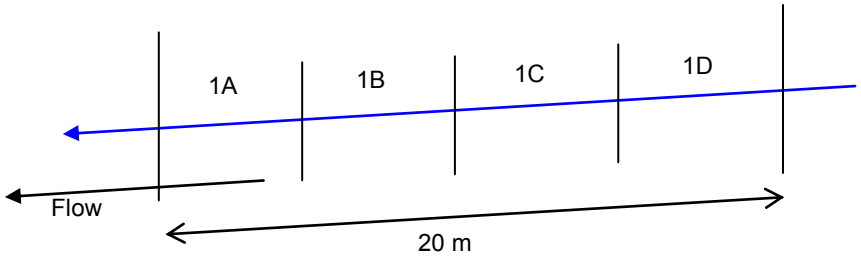


Figure 2.7. Illustration of sub-site labelling

- The distance between sites (of 100 m) was selected to minimise the impact on water quality, such as increased sedimentation, occurring from sampling upstream. Distances between sites might be reduced, such as where total length of the tributary is less that 320 m or where sub-sites require relocation resulting from obstacles across the stream (see below). They might also be increased on first order streams where overall stream length exceeds 350 m.

- To distinguish between sampling events (each site sampled four times), each sub-site would be sampled once, sequentially in an upstream direction from sub-site A to sub-site D (e.g. 2ACa1, 2BCa1 etc). Randomisation of sub-sites would not be implemented due to the potential for sampling to disturb sub-sites immediately downstream, as well as the removal of individuals that might otherwise colonise downstream sites. Upstream

colonisation is considered less likely, due to limited dispersal capabilities of *Beddomeia* from downstream (Spiers 2003). Sampling of sites would also be sequential in an upstream direction from site 1 to site 3 for similar reasons.

- Where obstacles such as flow diversion (underground flow) or large fallen trees across the stream affect the site selected using the distance selection method above, the site should be relocated in an upstream direction, in distance increments of 5 m until the channel had reformed and surface water flow resumed, or the obstacle has been cleared. Each 20 m site should be positioned such that it was free of these impediments (surface flow and no large tree limbs blocking access). Further alterations might be necessary over subsequent visits due to windthrow, flood and/or drought affecting the site.

2.5 Conclusion

Both the visual assessment and washing methods gathered useful data on hydrobiids; however, rock washing followed by microscopic examination provided the more accurate data (summary table).

Table 2.7. Summary of advantages and disadvantages of the Visual Assessment (VA) and Washing (WM) sampling methods.

Issues	VA	WM
Accuracy & Precision	Inaccurate (75%) Low precision	High field sampling accuracy Low precision amongst field staff High precision per individual
Sampling time	Limited by length – detail relationship. Longer than WM	Short field time, but lengthy lab component
Sampling effort	Training required. (lack of consistency and accuracy with untrained personnel) Limited scope for sampling large volumes.	Low level skill required for field sampling, but consistency required. Able to sample large volumes of material in short time
Population data	Restricted to adult/juvenile and breeding (presence of egg capsules)	Good (6 categories), higher counts. Inconsistent detection of breeding
Morphotypes	Unable to detect	Able to detect
Invertebrates	No by-catch	By-catch data available, providing supporting evidence for mollusc data
Threatened species	Minimal impact	High impact

Provided the researcher has the time to survey and process samples, the washing method provides more scope to obtain meaningful population structure and abundance data, as well as sympatric species and associated by-catch community data.

Identified variation in snail abundances and densities suggested changes to the method and design protocol for a spatial and temporal catchment-wide investigation. Error margins were recognized in the method, however provided suitably trained field staff conduct the sampling, these are considered sufficiently low to detect real changes in population densities and structure.

Changes in the methods to be incorporated into the sampling protocol were identified and include a reduction in site length, standardization of amount of habitat surveyed, and a third habitat type of potential habitat identified to be included in the study.

2.6 Appendix

ANOVA and parameter estimates of *Beddomeia* abundance against position in stream.

Source	DF	SS	MS	*F* Ratio
Model	1	41184.500	41184.5	95.0046
Error	1	433.500	433.5	Prob > F
C. Total	2	41618.000		0.0651

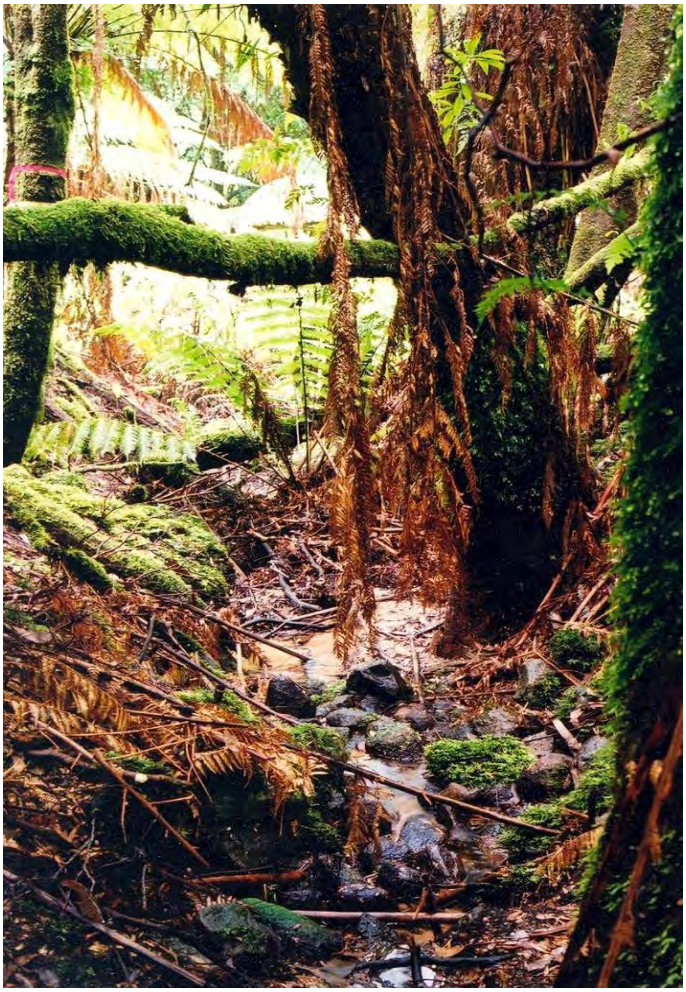
Term	Estimate	SE	*t* Ratio	*p*>|*t*|
Intercept	-52	31.80409	-1.64	0.3495
Site	143.5	14.72243	9.75	0.0651

Chapter 3

An Ecological Investigation of *Beddomeia* species

Quote: "*It's very pretty [in the forest] when you stand still*" J. Meggs (April 2002)
Quote "*There's a bit of coarse woody debris [CPOM] that would melt your heart!*" Qug (Mar 2003)

Chapter 3 presents an ecological study on the habitat selection and distribution of *Beddomeia* spp. across two catchments at temporal and multiple spatial scales. Habitat parameters were examined at the smallest scale, and population densities and structure in relation to location within catchments were investigated.



Headwater stream, Groom River catchment, Blue Tier

3 The distribution and habitat characteristics of *Beddomeia* (Hydrobiidae: Mollusca) in production forest within two catchments in northern Tasmania

3.1 Abstract

Patterns of spatial and temporal distribution of two narrow-range endemic species of *Beddomeia* were investigated across two major river catchments (Castra Rivulet and Groom River) in northern Tasmania between 2001 and 2004. The results indicate that the *Beddomeia* species are headwater stream specialists and that their response to anthropogenic disturbance varies with species and the type of disturbance. Sympatrically occurring morphotypes of *Beddomeia* were recognised in each catchment, displaying similar patterns of abundance for specific habitats in headwater streams, where proportionally, the most breeding was found to occur. Habitat variables influencing *Beddomeia* presence and abundance differed between catchments. Geology, catchment size, altitude, flow and forest community were factors influencing *Beddomeia* presence and abundance in the Castra catchment. Catchment size, disturbance gradient and forest community were, however, the main factors influencing *Beddomeia* abundance in the Groom River catchment. Snail abundance was found to decrease with increasing catchment size in both catchments, however influence of disturbance, which was also negatively correlated with snail abundance, was identified as unique to the Groom River catchment.

Range restrictions and specific habitat requirements are features of narrow-range endemic species. We suggest that improved measures may be necessary to reduce disturbance to the upper reaches of small streams where such narrow-range endemics species occur. It is recognised, however, that additional research investigating aspects of habitat modification on freshwater molluscs, in particular high impact forestry and agricultural clearing, is required to quantify the effects.

3.2 Introduction

The freshwater gastropod family Hydrobiidae, which is found across the globe; is considerably diverse, to date around 400 genera are recognised in the family (Kabat and Hershler 1993). While the number of native hydrobiid genera found in Australia is relatively modest, areas of rapid species diversification exist in the east and south. Several large radiations occur, including one, *Beddomeia*, endemic to Tasmania, containing 46 named species, and four subspecies. Short-range allopatric speciation is a feature of organisms with limited dispersal capabilities, small size, limited fecundity and particular habitat requirements, features illustrated by the *Beddomeia* genus (Ponder *et al.* 1993, Ponder and Colgan 2002). Molecular techniques have recently been used to validate these radiations of hydrobiids, or closely related families, both in Australia (Ponder *et al.* 1994, Ponder *et al.* 1995, Clark *et al.* 2003, Perez *et al.* 2005) and the U.S.A. (e.g. Liu *et al.* 2003, Hershler and Liu 2004, Liu and Hershler 2004, Hershler *et al.* 2008), confirming in at least a few cases, a more diverse freshwater molluscan fauna at the spring, stream or catchment scale than was previously thought. A systematics study conducted on a subset of Tasmania's hydrobiids suggests that this is also the case in the Tasmanian hydrobiid fauna (Chapter 6).

Distributional studies of various hydrobiid genera, or genera of closely related families, have been conducted in various parts of the world (e.g. Hershler 1999, Lydeard *et al.* 2000, Liu and Hershler 2005, Hershler *et al.* 2008), including Australia (Ponder and Clark 1990, Ponder *et al.* 1993, Ponder *et al.* 1995, Clark *et al.* 2003, Perez *et al.* 2005, Ponder *et al.* 2005). Others have reviewed the invasion of introduced hydrobiid species such as *Potamopyrgus antipodarum* in Greece (Radea *et al.* 2008), California (e.g. Herbst *et al.* 2008) and across Australia (e.g. Ponder 1988, Loo *et al.* 2007a, Loo *et al.* 2007b); Alonso & Castro-Dìez (2008) discuss the invasion success of *P. antipodarum*. However, few researchers have investigated the habitat requirements of hydrobiid snails, generally recording only limited environmental data, while investigating specific effects such as salinity and temperature on egestion on *Hydrobia* spp. (Hylleberg 1975), or else recording habitat variables from a single observation at each site (e.g. Ponder *et al.* 1994). Notable recent exceptions are the studies by Sada (2001, Sada *et al.* 2005, Sada 2008) which provide detailed reviews of environmental factors influencing the structure of an assemblage of native spring snails in Nevada, U.S.A. Most other studies, however, have inferred ecological preferences from collection locations such as: „on rocks and leaf litter' (Ponder *et al.* 1993, Clark *et al.* 2003); from depth (e.g. Ponder *et al.* 1993, Davies and Cook 2002); or specific sites such as hot water springs (Ponder *et al.* 1989, Ponder and Clark 1990, Ponder *et al.*

1995, Sada *et al.* 2005). Detailed examinations of microhabitat preference and broader ecological requirements are scant or missing from the literature (Ponder and Colgan 2002).

Beddomeia spp. are found almost exclusively in flowing water, ranging from springs to major rivers; many species in the genus have restricted distributions, being recorded from only single sites or small streams (Ponder *et al.* 1993, Ponder 1996). Despite their diversity and often high abundances in small streams, few ecological studies have been undertaken on Tasmania's hydrobiid fauna. Since the taxonomic review of Ponder *et al.* (1993) only two other, unpublished, studies have been conducted on *Beddomeia*, both relating to *Beddomeia launcestonensis*, a riverine species from the lower reaches of the South Esk River (Davies and Cook 2002, Spiers 2003 (Hons thesis)). These studies provide information on the critical habitats and aspects of feeding preferences, phototaxis, and behaviour in flows, in reference to shell morphology, affecting the distribution of *B. launcestonensis*, but how this relates to the majority of *Beddomeia* spp. inhabiting other environments such as small headwater streams is uncertain.

Many species of *Beddomeia* occupy narrow ranges that coincide with areas subjected to either forest harvesting or agriculture (Ponder *et al.* 1993, Ponder 1997a, Forest Practices Board 2001). Issues related to the conservation of narrow-range endemic freshwater snails are highlighted in papers such as Ponder (1997b) and Ponder & Colgan (2002); in recognition of limited available information on patterns of speciation, perceived threatening processes such as forestry, mining and dam construction, combined with poor conservation efforts protecting habitat of these species.

This study examines the spatial and temporal distribution, abundance variability and population structure of *Beddomeia* species in two river catchments in northern Tasmania. It also aims to identify and compare habitat preferences within the river catchments and to investigate the relationship between habitat variables, including disturbance, and the occurrence and abundance of *Beddomeia.*

3.3 Methods

3.3.1 Site selection

Two catchments in northern Tasmania, Castra Rivulet and Groom River were selected for this study since *Beddomeia* spp. were known to occupy at least one stream in each catchment. Approximate locations of catchments and sites are presented in Figures 3.1*a, b*.

The Castra Rivulet – Deep Gully Creek catchment, is a deeply incised, native forest gully, dominated by wet *Eucalyptus obliqua* forest, set in a fragmented rural landscape located primarily within the Castra State Forest, Upper Castra, with an altitudinal gradient of 120 to 530 m above sea level. Sections of the catchment are highly modified by agricultural clearing (~ 40%) and plantation establishment (~ 10%), the majority of plantations established since 1999. The catchment is bisected by a change in geology from basalt, with deep, rich red/orange soils in the eastern section, to dominantly felsic volcanosedimentary sequences of sandstone, siltstone, conglomerate, volcaniclastic breccia and minor lava, with flaky, angular rocks and gravel in the western section. Higher altitudes within the catchment coincide with the geological change from basalt (lower catchment) to siltstone, while a transition zone approximately 1 km wide exists within which a number of study streams show signs of a mixed geology.

The Groom River catchment is situated within the Goulds Country State Forest, west of St Helens in northeast Tasmania. The altitudinal gradient of this catchment is between 120 and 850 m. Land clearing for agriculture within the catchment is confined to 5% of the total area. The remainder is principally *Eucalyptus regnans* dominated State forest, of which < 2% is *Eucalyptus nitens* plantation, established in 2003, and a further 3% has been native-forest harvested since 2001. Tributaries of this catchment flow though a homogeneous geology dominated by adamellite/granite and associated dykes or alkali-feldspar granite.

A nested-sampling design employing the protocol established in Chapter 2, was used, incorporating a total of thirty-six streams across the two catchments: 18 streams were selected per catchment, each containing three, 20 m sites divided into four x 5 m sub-sites, except for two streams in the Castra catchment which had only two sites (Ca8 and Ca11) (Figure 3.1*a*). Stream locations were selected on the basis of accessibility and stream order (Strahler 1957). Where available and accessible, replicate streams of each stream order were incorporated into the design (Table 3.1). Sites at each stream location were separated by a

minimum distance of 100 m along each stream, numbered such that site 1 was most downstream, within 20 m of the confluence with another stream, and site 3 was most upstream. Location of study sites is presented in (Appendix A).

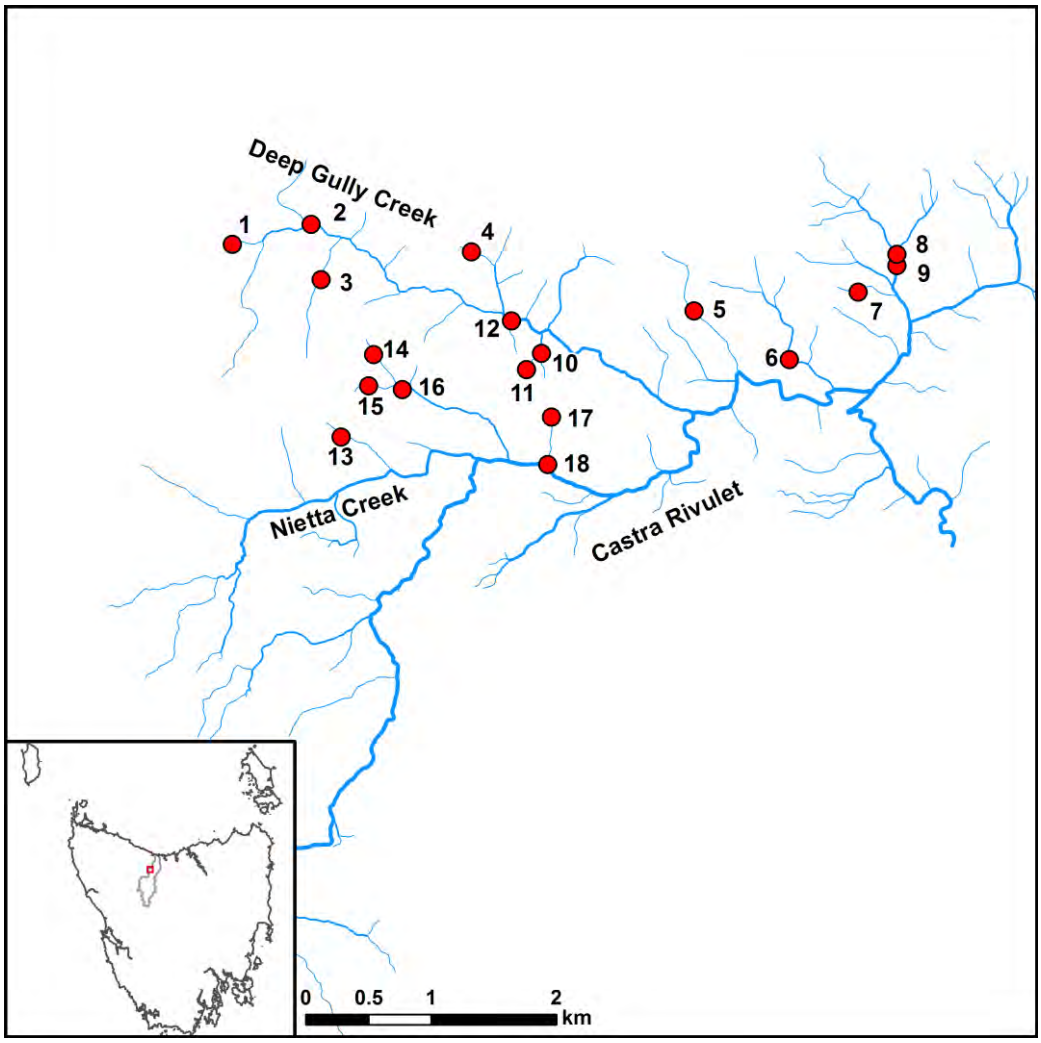


Figure 3.1*a*. Castra catchment study streams. Filled circles indicate the location of each study stream. (Note stream 8 is located on the western fork of the upstream catchment).

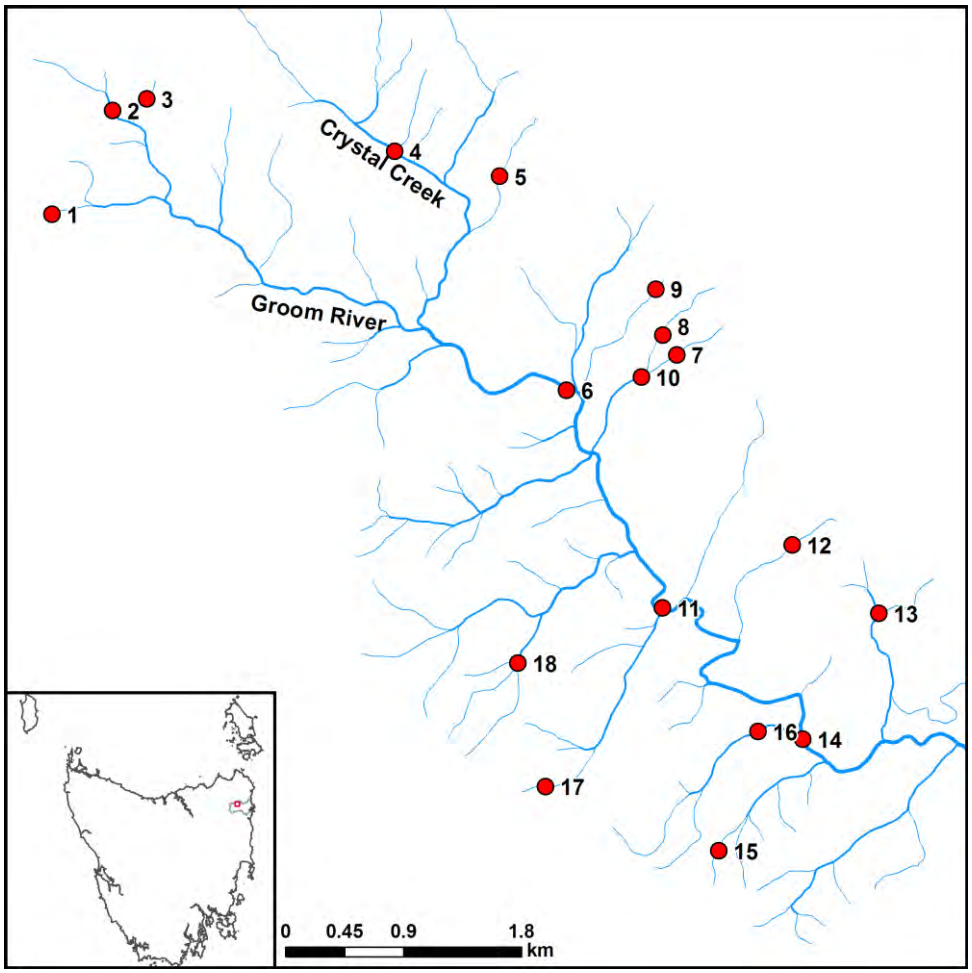


Figure 3.1*b*. Groom River catchment study streams. Filled circles indicate the location of each stream.

Stream catchment sizes, at site 1 (catchment data combined), ranged from 3.2 ha to 2656 ha, with the minimum catchment size at any single sub-site (at site 3) recorded as 0.7 ha. The ranges of catchment sizes above site 1 for different stream orders are presented in Table 3.1. Castra Rivulet was the smaller of the two catchments, having a catchment size at the fifth order stream, site 1Ca18 (on Castra Rivulet) of 850 ha, compared to the equivalent site, 1GC14 on Groom River at 2656 ha. Given the nature of catchments (multiple headwater streams flowing into progressively fewer, larger streams) the number of streams replicated in this study is skewed toward smaller headwater streams, since fewer larger streams were available within each catchment, resulting in unequal replicates in this study. The number of streams sampled per stream order is shown in Table 3.1. Replication of stream order data was introduced with successive sampling of sub-sites (sites x 4).

Table 3.1. Number of stream orders sampled per catchment and range of catchment sizes associated with each stream order.

stream order*	Castra			Groom River		
	streams	sites	catchment size (ha)**	streams	sites	catchment size (ha)**
1	6	23	5.6 – 32.5	6	23	3.2 – 18.6
2	9	20	13.3 – 98.4	7	16	14.4 – 95.8
3	1	3	106	2	8	2834 – 110
4	1	3	850	1	1	169
5	1	3	396	2	6	1941 - 2656
Total	**18**	**52**		**18**	**54**	

* based on GIS Tasmap (Zone55) 1:25000 scale topographic map
** at site 1 on each stream

3.3.2 Sites sampled

Equal numbers of study streams were selected in the study catchments, but the number of sites and sub-sites sampled differed due to some sites being ephemeral (usually site 3) and length of stream; the length of one Castra stream permitted only two sites, the other was dry at site 3 after the first sampling season. In addition, where *Beddomeia* spp. were not recorded from any site on given streams during the first two sampling events, sampling of the stream was discontinued. One stream in the Groom River catchment was also discontinued because of the absence of snails after sampling season 1. Lack of availability or low abundances of habitat, produced further divergence in the numbers of individual habitat type samples as well as the total number of samples collected per catchment. Within the Castra catchment the total number of sub-sites visited was 184; from which 518 habitat samples were derived while 36 habitat samples were not taken. This compares with the 202 sub-sites sampled within the Groom River catchment from which 554 habitat samples were obtained and 52 individual habitat samples not collected.

3.3.3 Fauna sampling

A total of four field visits were undertaken, during late summer - autumn (February to April) and spring (September to November) between September 2001 and May 2003. Sites were visited four times, with each sub-site sampled once. An ordered sampling design was employed to sample sub-sites, rather than a random method, with sub-site A (most downstream) sampled during the first season, through to sub-site D (uppermost) on the final

visit. This approach was used to reduce the possible impacts that upstream sampling may have on the natural habitat availability (amount of natural CPOM in-stream) and to remove any potential alteration to sedimentation caused by previous sampling events.

Three habitat types (rocks, woody debris or coarse particulate organic matter including leaves, twigs, branches and bark (CPOM) and streambed substrate (substrate)) were sampled at each sub-site, where available, using the „washing method' developed in Chapter 2, and sub-site data recorded. The presence of *Beddomeia* egg capsules, where visible on the habitat material during processing, were also recorded. Sampling was conducted using a semi-quantitative sampling methodology, with constraints placed on the amounts of habitat types sampled. This was restricted to one tray of 36 x 26 x 5 cm for CPOM; 52 rocks of proportions: (25 pebbles (< 6 cm) + 25 cobbles (> 6 < 25 cm) + 2 boulders (>25 cm) diameter); and 5 x 200 ml containers of stream substrate and habitat sampling time per habitat type limited to 20 minutes. Within each 5 m sub-site, sampling area was confined to a 5 x 2 m section; such that the entire channel of smaller streams (stream order 1-3) could be sampled but in larger streams (stream order 4-5) the area was restricted to a width of 1 m along each bank within sub-sites. The „washing method' was applied to sampling of rock and CPOM habitats, whereby each habitat type was washed in a separate bucket of water, the habitat surfaces agitated by hand to remove molluscs and associated macroinvertebrates. The bucket contents were then sieved through a 300 µm mesh net and the contents transferred into 200 ml containers and preserved in 5% formalin, or 70 % ethanol. Substrate was sampled by removing five 200 ml jars of stream bed substrate from an area of 50 x 50 cm of stream bed. Each container was lowered, mouth-side down, onto the substrate and material scooped into the container from beneath, whilst simultaneously holding an open 300 µm mesh net immediately downstream to catch any disturbed substrate. Container and net contents were then sieved through another 300 µm mesh net and the contents transferred into a 500 ml container and preserved in 70% ethanol.

Samples were processed in the laboratory with the aid of a Leica MZ75 stereo dissecting microscope. Aquatic molluscs were identified to species and counted, while *Beddomeia* spp. were further classified into morphotype, based on microscopic examination of external shell characteristics (described in Ponder *et al.* 1993, Hershler and Ponder 1998, and Chapter 5) and counted. Morphotypes were also categorized into size class, (Adult, S1 – S4, Juvenile), separated on number of whorls to obtain population structure data. Snail abundances, densities (no. cm^{-2} of rock surface and no. per tray of CPOM) and population age structure

were obtained for each substrate type at each site. *Beddomeia* egg capsule presence on material collected was recorded and habitat type noted.

3.3.4 Habitat variables

Habitat variables recorded at each site and used in the final analysis (Table 3.2), and were chosen for their anticipated value as predictors of *Beddomeia* spp. distribution and abundance, and for the ease with which they could be collected.

Streambed structure

Composition of sub-site streambed was recorded at each sub-site, with length divided into percentage of pool, riffle and run, and substrate type categorized into percentage of bedrock, boulders, cobbles, pebbles, gravel, sand, mud, and other (e.g. tree fern roots).

Disturbance grade

Disturbances within the Castra catchment mainly stem from a combination of historic and current agricultural clearing and forestry activities (e.g. historic selective harvesting, *Pinus radiata* plantation establishment and recent 2nd rotation harvesting, and more recently, *Eucalyptus nitens* plantation establishment on previously cleared land).

Historically, the Blue Tier, in which the Groom River catchment has its origins, was heavily impacted by tin mining operations that began during the late 1870's. Due to dwindling tin resources, most of the operations had ceased by the 1920's, however one, the Anchor Creek mine, continued periodically operating through to the 1990's (Lesser 1987, Jackman 1998). Additional historic disturbances to the catchment included: agricultural and settlement clearing, selective harvesting, and a severe fire event in the early 1900's. More recently, some clearfall forestry operations have been conducted within the catchment, and agricultural pursuits in the lower catchment continue to impact on the Groom River. For the purpose of this study, and to limit potential bias in the analyses, historic mining disturbance was considered a catchment-wide phenomenon and given a set disturbance level, upon which other disturbances were added (Table 3.3 and key to table presented in Appendix B). This is supported by historical photographic evidence of the Lottah, Blue Tier and Anchor Ck mining area (e.g. Figure 3.2), showing near complete forest cover removal and extensive stream channel modifications, and from mineral and mining lease charts at the Department of Mineral Resources Tasmania (Jackman 1998, St Helens Historical Society). An example of this landscape modification in one section of the catchment is shown in Figure 3.2, where

locations of streams GC8 and GC9, situated in the gully upstream of the Anchor Tin Mine, are represented by the red arrows.

Figure 3.2. Historic postcard illustrating environmental changes resulting from the Anchor Tin Mine operation at Lottah, *c.*1900 (courtesy St. Helens History Room). Red arrows indicate approximate location of streams GC8 (left) and GC9 (right).

Table 3.2. List of habitat variables included in final models.

Variable	Treatment
Floristic community	11 floristic categories were used (O10, PPR, RC1,.1, RC1.2, SD6, SE1, SE1-2, SE1-3, SE1-4, WO0110, WR101)
Sampling event	Sampling season (1 to 4) and year
Geology	Four geology types were recognised (S, O, B, G)
Coarse rocky substrate	= combination of bedrock, boulders, cobbles and pebbles. Coarse rocky substrate and fine rocky substrate add to 100%, therefore fine rocky substrate was omitted
Flow data	Transformed using Log_eflow
Catchment data	Transformed area using Log of catchment size
Disturbance grade	A scale measure of disturbance to catchment at sub-site (and above). Includes historical disturbance (see Table of Disturbance categories). *(grading scale between 0 to 5)*
Altitude	In metres above sea level, from 1:25 000 topographic maps.
Slope	Degrees
CPOM instream	Percentage of CPOM in sub-site
Depth	In cm
Width	In cm
Sub-site structure	Percentage of pool and riffle per sub-site
Stream substrate	Percentage of boulders, cobbles and pebbles
Riparian slope 1 & 2	slope in degrees at right angle to stream
Aspect	Direction of slope in degrees
Site composition	Run, riffle and pool. Recorded as percentage of site and add up to 100%, therefore percentage run was omitted
Canopy cover	Arcsine square-root transformed owing to preponderance of high and low values on a percentage scale. Average measurement (% cover) of the stream canopy cover.*(from 4 measurements taken per sub-site using a Densiometer)* (Lemmon 1957)

** The floristic community code = (Floristic community code & RFA community name)
O10 = (OTHER-10 = *Acacia delbata* forest);

PPR = *Pinus radiata* plantation;

RC1.1 & RC1.2 = (RAIN-C1.1 & RAIN-C1.2 = Callidendrous & thamnic rainforest);

SD6 = (SWAMP-D6 = Callidendrous & thamnic rainforest - Riparian blackwood forest);

SE1, SE1 – 2 to SE1 – 4 = (SWAMP-E1 - E4 = depauperate montane tea-tree forest);

WO0110 = (WET-OB0110 = Wet *Eucalyptus obliqua* forest);

WR101 = (WET-REG101 = Wet *Eucalyptus regnans* forest) &

WR110 = (WET-REG110 = Wet *Eucalyptus regnans* forest). Taken from (Forest Practices Authority 2005)

Most streams sampled in this study showed some level of human impact, with only four streams in the Castra system undisturbed by human activity (natural disturbance only), while all but one stream in the Groom River catchment showed direct evidence of historic mining disturbance. Therefore, a disturbance category was constructed for each of the streams, with levels of categories ranging from natural (0) to heavily impacted (5) with increasing amounts of harvesting, agricultural impacts and historic mining influences attributed to higher

categories. The descriptions of the disturbance categories attributed to each of the streams within the study are presented in Table 3.3, and the key to the table in Appendix B.

Water quality parameters

Dissolved oxygen, electrical conductivity (EC), pH and temperature were measured using WTW microprocessor oximeter OX196 (Zoology # 3-94) and WTW microprocessor conductivity meter LF 196 (Zoology #1). Flow measurements were recorded using two methods: a miniature propeller flow meter (MiniWater2 Micro 661/22, Schiltknecht Messtechnik, Gossau, Switzerland) was used to determine flow velocities (for one season), while surface flow estimates were used on successive visits. Surface flow was estimated using measurements of time taken for a floating object (an empty 10 ml vial) to cover 5 m of stream. Four measurements were taken per sub-site, and average flow estimates reported in $m.sec^{-1}$. Dissolved oxygen, pH and EC were recorded at each location during the first two sampling seasons, but were discontinued due to lack of variability across individual catchments. Temperature and turbidity data were collected for all four seasons.

Table 3.3. Disturbance classification based on types and levels of disturbance within each catchment (see Appendix B for explanation of disturbance grade and degree of disturbance).

Stream	Disturbance type and % of catchment disturbance	Degree of disturbance	Disturbance grade
Ca1	Pine plantation at 2 sites and minimal riparian vegetation present. Roading. Native riparian forest at third site	1b, 4a,c	3.4
Ca2	Road parallel to two sites. Rubbish dumped. Road culverts draining into stream	4b	2.5
Ca3	Coupe on one side of stream harvested, however, wide riparian buffer (> 30 m). Intact riparian zone	0.5	0.5
Ca4	Upper catchment cleared for agriculture (historic), forest partially harvested nr stream. High percentage of weeds (blackberries)	1g, 3a, 4c, 4b	4
Ca5	>50%; harvested within State forest and private land	1h, 4c	3
Ca6	>60% harvested within State forest and private land	1h, 4c	4
Ca7	Stream in unharvested forest > 100 m from disturbance	0	0
Ca8	Upstream agricultural clearing, some clearing of forest adjacent to stream (patchy)	1b, 4c	2
Ca9	Most of catchment above site cleared for agriculture	Upper 3, 6	2.5
Ca10	Nil	0	0
Ca11	5% of upper slope above headwater cleared, but > 100 m away	0	0.5
Ca12	Some tributaries harvested. Major road through, rubbish present	4, 1, 3	2
Ca13	Partial catchment clearfall – Plantation est. good riparian buffers	1h, 4c, 5c	4.5
Ca14	Riparian buffer intact, regenerating after burn, within a recently harvested forest operation. Road over headwaters of stream.	1b, 4c	4
Ca15	Riparian buffer intact at two sites, third site regenerating after burn, within a recently harvested forest operation. Tracks over headwaters of stream	1b, 4c	4
Ca16	In intact forest, but downstream of streams 14 and 15	1b, 4c	2
Ca17	Pine plantation, no riparian buffer	1b, 4a, 6	5
Ca18	Accumulated disturbances from land clearing and forestry operations conducted within the catchment	Some 1b, 3b	3
GC1	85% disturbance from mining and land clearing	2a,b	5
GC2	60% disturbance from mining. Excellent riparian buffer	2b	3
GC3	70% disturbance from mining, land clearing (at Blue Tier)	2a,b, 3a – upper	4
GC4	70 – 80% -disturbance from mining	2a,b,c	4.5
GC5	100% disturbance from mining	2a, 5a, 6	4.5
GC6	100% disturbance from mining	2a, 5a, 6	4.5
GC7	40% upper catchment undisturbed (small area)	1h, 2b	2
GC8	70% upper catchment possibly undisturbed (small area)	2a, 4a	2
GC9	70% upper catchment possibly undisturbed (small area)	2a, 4a	2
GC10	70% upper catchment possibly undisturbed (small area)	1h, 2a, 4a	3
GC11	Accumulated disturbances from land clearing, mining and forestry in upper catchment	1h, 2b,c, 3a,b, 4a, 5c	3.4
GC12	Upper 10% agriculture, upper 30% historic mining	2a – upper, 2b	4
GC13	80% disturbance: 25% agriculture; historic mining	2, 3	4
GC14	Accumulated disturbances from land clearing, mining and forestry in upper catchment	1h, 2b,c, 3a,b, 4a, 5c	3.4
GC15	20% disturbance: upper catchment retained. Main highway crosses headwaters. In unharvested forest	4b, 6	1
GC16	Defined riparian zone. Upper 30% agriculture, the rest selectively logged (historic) and Mining activity (historic)	3b – upper, 4b – upper, 5c	2
GC17	20% disturbance from historic mining and forestry activities in upper catchment	4a	1.5
GC18	60% Disturbance from mining, selective harvesting (historic)	2a,b	3

3.3.5 Data Analyses

To address the question of the spatial and temporal distribution of *Beddomeia* spp. at the catchment level, the response variable „*Beddomeia* abundance' was first analysed against the full environmental variable dataset to screen out redundant variables, and subsequently a subset of the environmental variables was analysed. Separate analyses were conducted for the density of *Beddomeia* spp. on each of the habitat types: CPOM, rocks and substrate, owing to differences in surface areas of habitat types and sampling method. Separate analyses were also conducted for each catchment as species to avoid issues associated with comparing different species.

After initial data inspection, a subset of habitat variables (see Table 3.2) were used to fit a full model using $\log(y + 0.1)$ transformed abundance of *Beddomeia* as the response variable. The transformation used was generally successful in ensuring the assumptions of multiple linear regression were met when plots of the standard diagnostics were inspected (Harrell 2001, Quinn and Keough 2002). There was some evidence of „zero-inflated' data (i.e. a large number of zero abundances), but this problem was most acute for the „substrate' habitat, where abundances were generally much lower.

The full model for each subset of the data was reduced using backward elimination; Akaike's Information Criterion (AIC) was used as the stopping rule: i.e. the final model had the lowest value of AIC in the model set. The algorithm used was that of Lawless and Singhal (1978) as implemented in the Design package of Harrell (2009) for R (R Development CoreTeam. 2009), and the fitting strategy is more fully described in Harrell (2001).

The issue of overfitting and biased selection of variables by the backward elimination procedures was addressed by bootstrap validation (Harrell 2001) ($p = 0.0001$), and in all cases the variables used in the final reduced models were those most frequently selected from the bootstrapped samples, and the results of these validations are not shown here for brevity. Accordingly, the final models presented here are likely to be robust while being parsimonious (Harrell 2001).

3.4 Results

3.4.1 Distribution and population structure of Beddomeia

Beddomeia species and undescribed morphotypes were present in 17 streams in the Groom River, and 14 Castra Rivulet streams respectively (31 of the 36 streams surveyed, Table 3.4). Each morphotype was recorded at multiple locations within each catchment; a total of four morphotypes (based on visual differentiation of shell features) occurred within Castra and another four, different morphotypes were present at Groom River. There were observable trends in morphotype abundances, and therefore dominance, within the catchments.

More than one morphotype was found in 27 of the 31 streams recording snails in the study. On average, three morphotypes occurred per stream in each catchment; the maximum number of morphotypes present at any site was four, occurring in 14 streams. A single morphotype occupied the remaining four streams, two per catchment; in each case these were the most common morphotype in the catchment (morphotype D at Castra and morphotype 6 at Groom River) (refer to Chapter 5 for descriptions). Patterns of dominance, as determined by the abundance of each morphotype per stream, differed between streams in each catchment (Table 3.5). Morphotype 6 dominated ten streams and was co-dominant in a further five streams in the Groom River catchment, while morphotypes 1 and 2 were each the most abundant morphotype in one stream. Determining dominance was further complicated by morphotype abundances changing between sites and sub-sites in individual streams. At Castra Rivulet, morphotype B dominated the snail population in seven of the streams, while morphotype D was most abundant in two streams, and B and D were co-dominant in five streams. Morphotype A was most abundant in one stream, although at the site scale, was the most abundant morphotype at several sites on three streams.

Snail abundance

Snail abundance varied throughout each catchment at all spatial scales; abundance negatively correlated with stream order. While the $p > F$ was significant (at $p > F = 0.0015$), the R^2 was low (at $R^2 = -0.154$) indicating a poor correlation. Snail abundance was also variable in first and second order streams, including between adjacent, or nearby streams, ranging between zero and 5500 snails (Figure 3.3).

Table 3.4. Presence of morphotypes per site *(Bold signifies dominant morphotype per stream)*. Total refers to number of streams in which each morphotype was recorded.

Stream	Morphotype				Stream	Morphotype			
	1	**2**	**3**	**6**		**A**	**B**	**C**	**D**
GC1	**1**	**1**	1	**1**	Ca1	0	0	0	0
GC2	1	1	1	**1**	Ca2	0	0	0	0
GC3	1	**1**	0	1	Ca3	0	0	0	0
GC4	0	0	0	0	Ca4	0	**1**	1	1
GC5	0	0	1	**1**	Ca5	0	**1**	0	1
GC6	0	0	0	**1**	Ca6	0	**1**	1	**1**
GC7	1	1	1	**1**	Ca7	1	**1**	1	1
GC8	1	1	1	**1**	Ca8	1	**1**	0	**1**
GC9	**1**	1	1	**1**	Ca9	**1**	1	0	1
GC10	1	1	1	**1**	Ca10	1	**1**	1	1
GC11	**1**	0	1	**1**	Ca11	0	**1**	1	**1**
GC12	**1**	1	0	**1**	Ca12	0	**1**	0	1
GC13	**1**	0	1	1	Ca13	0	0	0	0
GC14	0	0	0	**1**	Ca14	0	0	0	**1**
GC15	1	1	1	**1**	Ca15	0	0	0	**1**
GC16	1	1	1	**1**	Ca16	1	**1**	1	1
GC17	1	1	1	**1**	Ca17	1	**1**	1	**1**
GC18	**1**	1	1	**1**	Ca18	0	**1**	0	**1**
Total	**14**	**12**	**13**	**17**	**Total**	**6**	**12**	**7**	**14**

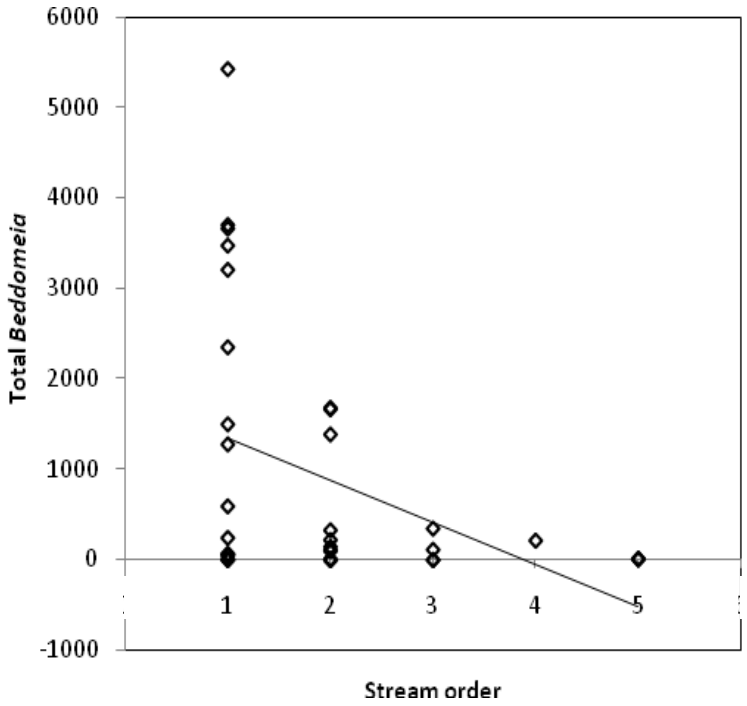


Figure 3.3. Total *Beddomeia* spp. per site by stream order for both catchments (data combined). Note that the data are skewed by single high value (135) in a fourth order stream (Ca18) on CPOM on a single occasion.

While this study did not directly investigate within-stream (between site) variation or habitat preferences, due to sampling method differences, within-stream influences of site were examined for individual stream order datasets, but no clear detectable pattern was observed. A general pattern of declining snail abundance in a downstream direction was present in some first and second order streams, however, there was too much variation in the data and no clear trend could be determined, nor could a trend between sub-sites be detected (Appendix A).

Direct comparison of the habitat type data (rocks, CPOM and substrate) to determine whether habitat preferences exist was also not possible; however a strong pattern of snail association with allochthonous material and rocks was evident in the headwaters of streams of both catchments (85.3% – 100%). Fine streambed substrate, although often used, did not appear to constitute important habitat for the *Beddomeia* species in this study; only one site harbouring 14.8% of snails, with the remainder retaining < 5% of snails.

Population structure

Six age cohorts were recognised in the snail populations of each river catchment. Despite significant variation in total abundances, the population structure of each morphotype showed a similar spread and composition of age classes. Due to this similarity, the population data for morphotypes was combined for analysis of each river catchment. No significant variation in the relative percentages of juveniles to other age cohorts was recognised at individual streams during this study. This finding is unsurprising, owing to the suspected longevity of *Beddomeia* spp. (greater than 5 years). Adults maintained at 10°C in captivity survived for a minimum of four years: (K. Richards unpublished data).

Groom River

Similar trends in population structure were observed across the lower order streams, with only minor differences in structure between some sites on individual streams (Figure 3.4). „Adults' contributed up to 40% of the population in the second order streams, averaging between 30 to 40% at most sites, and up to 45% in first order streams; juvenile and S4 individuals combined, made up less than 15% of the total population at most headwater stream (first and second order stream) sites, the exception being at GC15 and GC18 where juveniles accounted for 23% of the population.

Population structure was most stable (less temporal variability) at the upper most site (site 3) on first order streams, the exception being ephemeral sites. The population structure was more variable in second order streams. Low snail abundances and sample replication for third to fifth order streams made generalising about population structure in such streams unreliable.

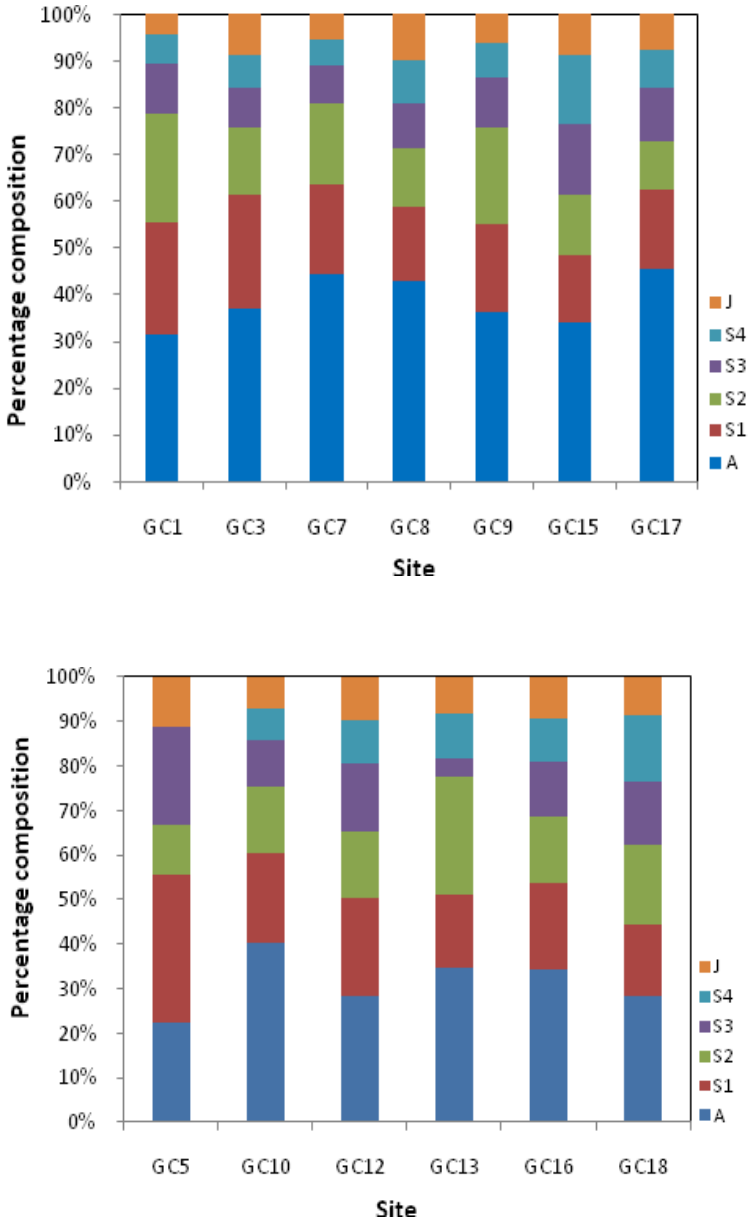


Figure 3.4. Total population structure of *Beddomeia* spp. in first and second order streams (habitats, sub-sites and sites combined) in the Groom River catchment. GC# refers to stream number. Population structure was separated into six size classes: J = juvenile, S1 – S3 progressively larger classes, S4 = subadult, and A = adult.

Castra Rivulet

The population structure observed in streams in the Castra catchment was highly variable. However, as observed at Groom River, adults contributed the greatest proportion to the

populations at most sites. Adults contributed between 20 to 40 % to the population in lower order streams, although at Ca5 adults and S1 cohorts made up 80% of the total (Figure 3.5).

At the site level, the population structure was found to vary between streams of the same order as well as between different orders with no consistent trends in population structure or abundance. Unlike the headwater streams at Groom River, no recognisable trend in population stability could be detected along stream gradients (between sites) at Castra. Interestingly, the highest proportion of juveniles per site was recorded in the largest stream (Ca18); however, caution is needed in interpreting the data due to the single data point, single habitat type (CPOM) and low snail abundances from which the data was derived.

Beddomeia egg capsules were recorded on all habitat types sampled from ten streams at Castra Rivulet and nine streams at Groom River. The majority of egg capsules were deposited on CPOM or rocks (refer to Chapter 5, Figures 5.2, 5.4*a, b*). Capsules were most abundant in upper catchment streams (headwater streams), the exception being a site on one fifth order stream in the Castra catchment from which a high proportion of egg capsules were collected on CPOM. Rocks and pebbles in headwater stream sites supported the highest numbers of egg capsules (Ca4, 17 and 10) in the Castra catchment, while CPOM and rocks recorded equally high numbers of capsules in small streams at Groom River. Egg capsules were only rarely encountered adhering to small substrate material such as granite gravel during laboratory analysis of samples (refer to Chapter 5, Figure 5.2*b*).

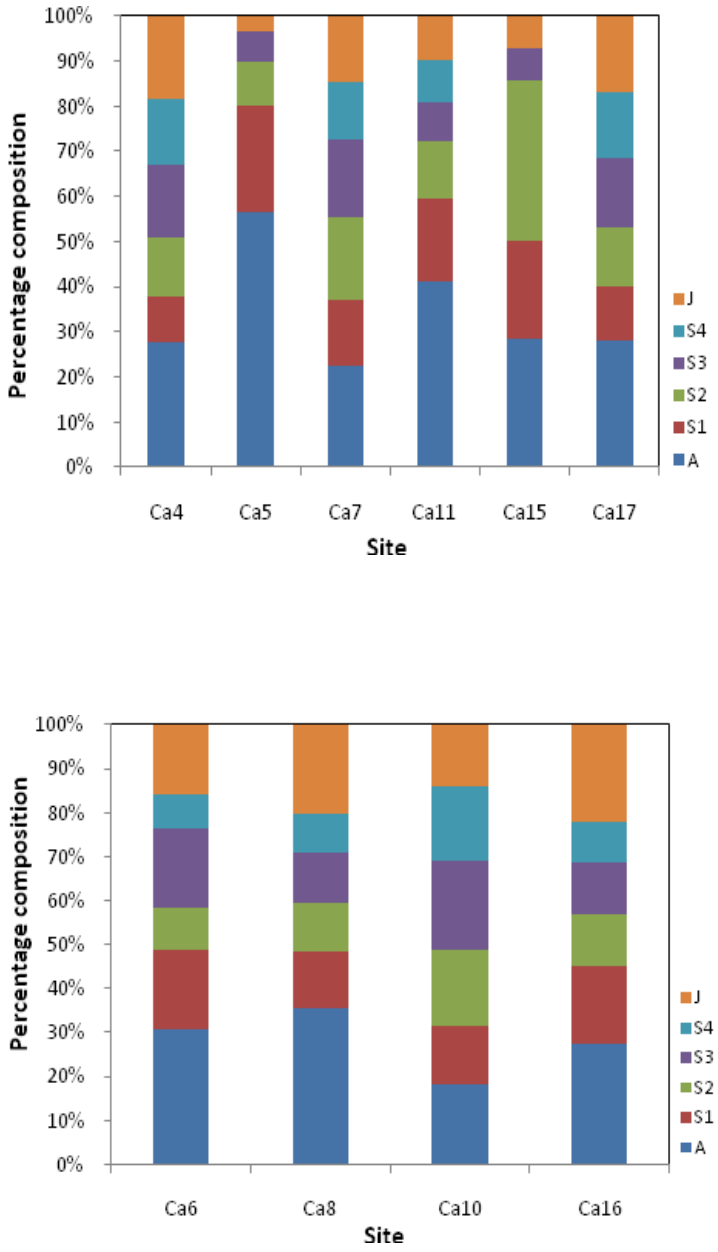


Figure 3.5. Total population structure of *Beddomeia* spp. in first (upper) and second (lower) order streams (habitats, sub-sites and sites combined) in the Castra Rivulet catchment.Ca# refers to stream number. Population structure was separated into six size classes: J = juvenile, S1 – S3 progressively larger classes, S4 = subadult, and A = adult.

3.4.2 Relationships between snail abundance and habitat variables

Groom River

The full model used in analyses for each subset of the data was:

log(*Beddomeia*+0.1) ~ altitude + aspect + slope.deg + logCatchment + disturbance.grade + riparian.slope.1 + riparian.slope.2 + coarse.rocky.subst + CPOM.instream + Depth + Width + log.flow + asn.canopy + fc + boulders + cobbles + pebbles + pc.pool + pc.riffle + season + yr.

Descriptions of each variable is presented in Table 3.4. fc = floristic community associated with riparian zone (Table 3.6); pc.pool = percentage of sub-site containing pools; pc.riffle = percentage of sub-site containing riffles; and yr = sampling event.

Rocks

The final model after backward elimination for „rock' habitat in the Groom River dataset identified catchment, disturbance grade, forest community and riparian slope.1 as most strongly correlated with the log of *Beddomeia* abundance (Table 3.5). The high R^2 indicated a reasonable model for estimating snail abundance.

Table 3.5. ANOVA of final model for Groom River rock habitat data. Response: log(*Beddomeia* + 0.1). Overall R^2 = 0.709, adjusted R^2 = 0.688, $F_{(11,153)}$ = 33.86, p <= 2.2 x 10^{-16}.

Factor	DF	Partial SS	MS	*F*	*P*
Catchment	1	106.787	106.787	48.02	<0.0001
Disturbance grade	1	114.262	114.262	51.38	<0.0001
fc	8	196.787	24.598	11.06	<0.0001
Riparian slope 1	1	22.525	22.525	10.13	0.0018
Regression	11	828.354	75.305	33.86	<0.0001
Error	153	340.235	2.2238		

Parameter estimates for the continuous variables (Appendix C, Table A) indicate a negative correlation between log snail abundance, catchment size (log catchment) and disturbance, the two most significant influencing factors (F = 48.02 and 51.38 respectively). The log value of snail abundance decreases by 0.649 for each unit of increase in log catchment size, and decreases by 1.13 for each unit of increase in disturbance. Increases in log snail

abundance were observed with increases in riparian slopes, although only marginally fewer snails were predicted, and only for one riparian slope. The ANOVA indicates the latter is the least influential of the variables retained in the model ($F = 10.13$, $p = 0.0018$).

For forest community, the log abundance of snails was greater than in streams with wet *Eucalyptus obliqua* forest (fcO10) in four forest communities: fcWR101, fcSE1-2, fcSE1, and to a lesser extent, fcSE1-3 (depauperate montane tea-tree forests 1-3).

CPOM

For CPOM in the Groom River, the final model retained catchment, disturbance grade, forest community and aspect as significant factors influencing the log of *Beddomeia* abundance (Table 3.6). There is less precision for this habitat than for ‚rocks' in this catchment, but over 50% of the variation in the data is explained by this model.

Table 3.6. ANOVA of final model for Groom River CPOM habitat data. Response: log(*Beddomeia* + 0.1) Overall $R^2 = 0.555$, adjusted $R^2 = 0.528$, $F_{(11,186)} = 21.06$, $p = < 2.2 \times 10^{-16}$.

Factor	DF	Partial SS	MS	*F*	*P*
Aspect	1	63.392	63.392	15.82	1e-04
Catchment	1	268.306	268.306	66.95	<0.0001
Disturbance grade	1	100.937	100.937	25.19	<0.0001
fc	8	167.358	20.919	5.22	<0.0001
Regression	11	928.329	84.394	21.06	<0.0001
Error	186	745.406	4.008		

This analysis suggests that disturbance (predominantly historic mining and clearing in this catchment) has a long-term influence on *Beddomeia* distribution and abundance.

Parameter estimates for the variables (Appendix C, Table B) indicate a negative correlation of log snail abundance with catchment size (log catchment), aspect and disturbance. Catchment size was the most influential factor, as revealed by the large *F*-value ($F = 66.95$), the log abundance of snails decreasing by 0.943 for each unit increase of catchment area. The relationship of snail abundance and disturbance level was negative, with an increase in log snail abundance of 1.013 with decreasing levels of disturbance, while aspect was

retained in the model, it is likely to be influenced by the lack of replication of streams and sites with an easterly or northerly direction of flow, so further survey work would need to be carried out to confirm the importance of this variable.

The community fcSE1 (depauperate montane tea-tree forest) supported many more snails (i.e. exp(3.257) = 26 times) than fcO10, with fcSE-2 and fcSE – 3 also supporting more than 7 times (i.e. exp(2)) the abundance of snails as fcO10.

Substrate

Only two variables were retained in the backward elimination for „substrate' habitat in the Groom River: log of catchment and forest community, although the modest R^2 shows that there is much residual variation in this model (Table 3.7).

Table 3.7. ANOVA of final model for Groom River substrate habitat data. Response: log(*Beddomeia* + 0.1) Overall R^2 = 0.363, adjusted R^2 = 0.334, $F_{(8,178)}$ = 12.66, p = 2.484 x 10^{-14}.

Factor	DF	Partial SS	MS	*F*	*P*
Catchment	1	45.821	45.821	20.89	<0.0001
fc	7	119.236	17.034	7.76	<0.0001
Regression	8	222.159	27.769	12.66	<0.0001
Error	178	390.513	2.194		

Parameter estimates for variables (Appendix C, Table C) indicate a negative correlation of log snail abundance with log of catchment size, the log of abundance of snails decreasing by 0.369 for each unit increase in log of catchment size. For the categorical variable forest community, the „SE' communities have more snails on average than wet *Eucalyptus obliqua* forest community „fcO10' as do fcSD6 and RC1.2 communities, the latter containing only marginally more snails.

Analysis of the Groom River catchment dataset (using the log (*Beddomeia* + 0.1) identified up to five significant explanatory variables (catchment size, disturbance grade, riparian slope, aspect and forest community) explaining the individual habitat type datasets. Overall, catchment size and disturbance category were highly significant explanatory variables (p < 0.0001), while the remainder were also important factors for various habitat types. Both

disturbance grade and catchment size were negatively correlated with *Beddomeia* abundance in the Groom River catchment, indicating lower snail abundance with increasing catchment size and disturbance gradient. Analysis of the habitat type subsets CPOM and rocks produced similar outcomes, differing only in one parameter (aspect replacing riparian slope for CPOM data). Two parameters were recognised as significant explanatory factors for substrate data (catchment size and forest community) but the final model had a low R^2 value.

Castra data

Geology was recognised as one of the most important explanatory variables for the Castra dataset using the transformation log (*Beddomeia* + 0.1) for the response variable (p = <0.0001). *Beddomeia* occurrence in streams containing siltstone geology was, at best, infrequent, with only one observation of snail egg capsules on the siltstone substrate; this was from a stream at the interface of geological change, where rocks of both geologies are present. Of the remaining significant explanatory variables, catchment size was negatively correlated with *Beddomeia* abundance for each habitat type as would be expected for a real effect, and together with the result for flow this suggests that the Castra *Beddomeia* are headwater specialists.

The full model used in analyses for each subset of the data was the same as for Groom River, but included geology, as this was variable across the study sites.

log(*Beddomeia*+0.1) ~ altitude + aspect + slope.deg + logCatchment + geology.scale + disturbance.grade + riparian.slope.1 + riparian.slope.2 + coarse.rocky.subst + CPOM.instream + Depth + Width + log.flow + asn.canopy + fc + boulders + cobbles + pebbles + pc.pool + pc.riffle + season + yr.

Rocks

The final model after backward elimination for „rock' habitat in the Castra Rivulet dataset identified catchment, geology, altitude, forest community and flow as most strongly correlated with the log of *Beddomeia* abundance and there are suggestive but inconclusive evidence of effects of log of flow and forest community effects (Table 3.8). The high R^2 indicated a reasonable model for estimating the snail abundance.

Parameter estimates for the continuous variables (Appendix C, Table D) indicate a negative correlation of log of snail abundance with altitude, catchment size (log catchment) and flow (log of flow). The two most significant influencing factors were catchment size and geology

(identified by the high $F = 32.65$ and $F = 16.70$ respectively). The log value of snail abundance decreases by 0.649 for each unit of increase in log catchment size.

Table 3.8. ANOVA of final model for Castra rock habitat data. Response: log(*Beddomeia* + 0.1). fc = forest community. Overall $R^2 = 0.606$, adjusted $R^2 = 0.581$, $F_{(10,157)} = 24.15$, $p = < 2.2 \times 10^{-16}$.

Factor	DF	Partial SS	MS	*F*	*P*
Altitude	1	26.151	26.151	9.72	0.0022
Catchment	1	87.804	87.804	32.65	<0.0001
Geology	2	89.824	44.912	16.70	<0.0001
Flow	1	21.055	21.055	7.83	0.0058
fc	5	40.541	8.1082	3.02	0.0126
Regression	10	649.562	64.956	24.15	<0.0001
Error	157	422.196	2.689		

For geology, the log abundance of snails was greater in streams with basalt than siltstone or the transition geology containing both siltstone and basalt rocks. The log abundance of snails declining by -2.49 for siltstone relative to basalt, and by -2.57 for the transition geology.

CPOM

For CPOM in the Castra Rivulet, the final model retained catchment, geology and forest community as significant factors influencing the log of *Beddomeia* abundance (Table 3.9). There is more precision for this habitat than for „rocks' in this catchment, with over 60% of the variation in the data is explained by this model. The high R^2 indicates a reasonable model for an ecological study.

Table 3.9. ANOVA of final model for Castra CPOM habitat data. Response: log(*Beddomeia* + 0.1). Overall $R^2 = 0.679$, adjusted $R^2 = 0.664$, $F_{(8,171)} = 45.29$, $p = < 2.2 \times 10^{-16}$.

Factor	DF	Partial SS	MS	*F*	*P*
Catchment	1	247.429	247.429	85.08	<0.0001
Geology	2	733.553	366.777	126.12	<0.0001
fc	5	104.021	20.804	7.15	<0.0001
Regression	8	1053.539	131.692	45.29	<0.0001
Error	171	497.280	2.908		

Parameter estimates for the variables (Appendix C, Table E) indicate a negative correlation of log snail abundance with catchment size (log catchment). Geology was the most influential factor, as revealed by the large *F*-value ($F = 126.12$), the log abundance of snails decreasing by 4.07 for siltstone relative to basalt and by -4.75 for the transition geology. Catchment was also an influential factor, as revealed by the large *F*-value ($F = 85.08$), the log value of snails decreasing by 0.897 for each unit increase in log of catchment size.

For the categorical variable forest community, the rainforest community RC1.2 supported more snails on average than fcO10 (i.e. exp(2.81) = 17 times more snails), and fcWR101 also supported more snails on average than fcO10 (i.e. exp(1.68) = 5.4 times more snails) while the other communities supported marginally more snails than fcO10, and fcWO0110 supported marginally less snails.

Substrate

Only two variables were retained in the backward elimination for „substrate' habitat in the Castra Rivulet: log of catchment and geology, although the modest R^2 shows that there is much residual variation in this model (Table 3.10).

Table 3.10. ANOVA of final model for Castra substrate habitat data. Response: log(*Beddomeia* + 0.1). The overall $R^2 = 0.471$, adjusted $R^2 = 0.462$, $F_{(3,160)} = 47.56$, $p = < 2.2 \times 10^{-16}$.

Factor	DF	Partial SS	MS	*F*	*P*
Catchment	1	67.948	67.948	37.52	<0.0001
Geology	2	198.059	99.029	54.69	<0.0001
Regression	3	258.366	86.122	47.56	<0.0001
Error	160	289.723	1.8108		

Parameter estimates for variables (Appendix C, Table F) indicate a negative correlation of log snail abundance with log of catchment size, the log of abundance of snails decreasing by 0.45 for each unit increase in log of catchment size, For the categorical variable geology, the siltstone geology has less snails on average than basalt soils, decreasing by 2.178 for siltstone relative to basalt and by 2.286 for transitional geology relative to basalt.

Only catchment size was recognised as an important descriptor of the *Beddomeia* distribution in both the Groom River and Castra Rivulet catchments where in both instances *Beddomeia* abundance was negatively correlated to catchment size.

3.4.3 Temporal changes

Direct comparison of sub-site data as a means of determining temporal changes in snail abundance was not possible because snails were removed during sampling, however, sub-sites within individual sites were used to determine trends in the population structure over time (included as season in the full model). Season was not found to influence *Beddomeia* abundance in any model. No trends in proportions of juveniles between spring and autumn sampling events were observed. This suggests that breeding is not seasonal, and that breeding occurs more regularly in headwater streams.

3.5 Discussion

3.5.1 Spatial and temporal distribution of Beddomeia

Considerable variability in *Beddomeia* distribution and abundance was observed throughout the two river catchments looked at in this study. Nevertheless the results clearly indicate that the *Beddomeia* species under investigation are headwater stream specialists, occurring in highest abundance in streams of small catchment size (first and second order streams) and occupying a high proportion of headwater streams sampled in each catchment.

The *Beddomeia* species investigated were most abundant in low stream flows, but able to tolerate a range of velocities (of 1.2 $m.s^{-1}$) for short periods, for example during flash-flood periods following heavy downpours. Headwater streams within the study catchments contained disproportionally high levels of CPOM composed of woody debris and high percentages of leaf matter. The limited ability of the headwater streams to move large substrate elements (rocks and CPOM) (Gooderham *et al.* 2007) and the use of these micro-habitats by snails is likely to partially explain high snail abundances in such streams. The complexity and volume of available CPOM varied between streams of different orders, changing from a complex combination of twigs, branch and bark material with a high proportion of leaves of multiple species in headwater streams, to a simpler matrix of increased fine particulate organic matter similar to that described by Gomi *et al.* (2002) in

third order streams. Larger woody material and occasional packs of broader leaves (*Eucalyptus* spp., musk (*Olearia argophylla*) and blackwood (*Acacia melanoxylon*)) wedged between boulders comprised the most abundant CPOM material downstream in fourth and fifth order streams. Behaviour of buoyant material such as leaves has been demonstrated to differ between species and with stream depth, and the length of time in suspension has been found to be dependent upon leaf species (Watson 2004). Leaf matter in headwater streams provides large surface area to volume ratios allowing for high levels of colonization by algae, and therefore snail presence. The lack of stream power to shift such debris allows time for periphyton to colonise leaves, thus providing more abundant foraging habitat for *Beddomeia* and other macroinvertebrates. The use of leaves as habitat in headwater streams and the proportionally smaller contribution of leaves in downstream CPOM partially explains the lower abundance of snails downstream. Despite the increased contribution of rocks, particularly boulders, to the streambed of larger streams, *Beddomeia* spp. were seldom recorded at such sites suggesting that either flow rates, depth or an interaction of these factors provide unfavourable conditions for snails.

While this study did not directly investigate within-stream (between site) variation or determine a habitat preference, due to sampling method differences, within-stream influences of site were detectable by examining individual stream order datasets. No clear pattern of declining snail abundance in a downstream direction was detected. Direct comparison of the habitat type data (rocks, CPOM and substrate) to determine whether habitat preferences exist was also not possible, however a strong pattern of perceived snail preference for allochthonous material and rocks was evident in the headwaters of streams of both catchments. Fine streambed substrate, although often used, did not appear to constitute important habitat for the *Beddomeia* species in this study.

The presence of high numbers of snails on both rocks and CPOM habitat types would seem to suggest it unlikely that periphyton levels differ substantially between these habitats, or that a dietary requirement unique to only one of these habitats exists. The fluctuating snail abundances is more likely a reflection of the availability and surface area of stable habitat.

As with many stream invertebrate studies, the sampling method applied did not allow for direct seasonal comparisons of snail abundances and population structure at a given sub-site due to the permanent removal of specimens. However, comparisons of immediately adjacent sub-sites within a given site enabled general observations of seasonal variation or temporal changes in *Beddomeia* abundance and population structure. Predictably, the total snail

abundance varied between sub-sites (at the same site); however, no significant differences at the sub-site level were detectable in either population structure or densities per habitat type between seasons. The lack of variation in young cohorts (juvenile and S4) within the population suggesting that no temporal variation occurs.

3.5.2 Relationship between snail abundance and habitat variables.

Environmental predictors of snail distribution and abundance were discernable at multiple spatial scales, from catchment to local levels. Previous ecological research on environmental requirements of aquatic spring snails in the U.S.A., suggest water temperature, current velocity, substrate type and channel structure (bank heights, angles and overhangs) are major factors contributing to the distribution of these snails (Sada 2001, 2008). The results obtained in this study, however, indicate that for these stream-inhabiting hydrobiid species, catchment size, flow, geology, altitude, forest community, aspect and disturbance are the major factors contributing to their distribution and abundance, although channel structure was not recorded in this study and therefore cannot be discounted.

The relationship between snail abundance and specific habitat variables in the current study was unique to each catchment. Altitude, geology, catchment size and flow were identified as influencing *Beddomeia* abundance in the Castra Rivulet catchment, although catchment size and geology contributed most to the model explaining the data. Within the Groom River catchment, catchment size, disturbance, forest community and aspect were the significant explanatory factors of snail distribution and abundance; however, disturbance was identified as a key explanatory variable in this catchment. Factors including flow and catchment size were negatively correlated with snail abundance, supporting findings of previous gastropod studies (e.g. Sada 2001, 2008), while the negative relationship between snail abundance and disturbance suggests that snails in some catchments may be sensitive to some types of anthropogenic disturbance.

Previous studies that report on the relationship between gastropod occurrence and stream flow have found that freshwater hydrobiids, and closely related families, are generally more abundant in low velocity flows (Ponder *et al.* 1989, Crowl and Schnell 1990, Collier and Winterbourn 2000, Richards *et al.* 2001, Spiers 2003, Sada 2008), while spring snails exhibit intolerance to fluctuations in temperature, salinity and dissolved oxygen and to desiccation (Ponder *et al.* 1989, O'Brien and Blinn 1999, Sada 2008). Davies & Cook (2002) and Spiers (2003) reporting on the distribution of *Beddomeia launcestonensis*, found this relationship

with flow to be equally true for this large-stream inhabiting species, but also noted that the species' habitat also experienced periods of energetic flows at times of extremely high river flows. *Beddomeia launcestonensis* occurs under large, stable rocks and boulders and in elevated scour pools. These apparent preferred habitat locations may reduce the impacts of extreme flows on the population, and the observation of minimal affects of flows up to 33 $cm.s^{-1}$, may account for the species continued presence in such environments (Ponder *et al.* 1993, Davies and Cook 2002, Spiers 2003). Several other *Beddomeia* spp. are also known to inhabit large streams, at least two species of which may be found under large boulders; however, their upstream range extent and habitat preferences remains unstudied (Ponder *et al.* 1993).

As catchment size increases so do water velocity, stream volume and depth downstream, and these variables also influence a stream's ability to retain CPOM and its composition, possibly the preferred habitat for *Beddomeia*. At the same time, a reduction in stream slope in larger streams alters substrate conditions and stable rocks become more prevalent (Gomi *et al.* 2002). Retention of such suitable in-stream micro-habitat also depends on the amount of overhanging riparian vegetation and hence the potential to create numerous small-scale debris dams and leaf packs (Gomi *et al.* 2002, Watson 2004). However, flow was only recognised as a contributing explanatory factor for the Castra data. A possible explanation for this is that the headwater streams in this catchment were, in general, steeper, thus the flows were greater and CPOM was more readily transported away during high rainfall events.

Geology was also identified as a major factor explaining snail distribution in the Castra Rivulet catchment. It is unfortunate that higher altitudes within the catchment correspond to a geological shift from basalt to siltstone, confounding the influences of the two variables, since this makes it difficult to determine the exact effects of each variable. *Beddomeia* was rarely found in streams on pure siltstone geology (coinciding with elevated parts of the catchment), and only one observation of reproduction was recorded on this substrate (and this was from a stream at the transition of the geological change, where rocks of both geologies occur). This result is contrary to the finding of limited difference in *Beddomeia* abundance on basalt and siltstone rocks recorded in Chapter 2, although those findings also came from a stream exhibiting transitional geology. However, the relationship is supported by observational evidence collected from adjacent catchments indicating that *Beddomeia* and *Austropyrgus* species occur at higher elevations in streams with basalt geology (K. Richards

unpublished data). The geology underlying the Groom River catchment is homogeneous and so contributes no further data to the relationship between occurrence and geology.

At least two explanations for the observed relationship between hydrobiids at Castra and geology (similar results observed in the *Austropyrgus* spp. data) are possible. Firstly, the different chemical composition of the geological substrates may limit the availability of calcium or other minerals directly to the snails. Secondly, differences in surface roughness may affect the snail's ability to adhere to rocks of the different geologies. Also, the mechanism making minerals available in the two substrates may change, altering the growth rate of the periphyton and bacteria on the different rock types. While these ideas need further investigation, the geological substrates at Castra do appear to influence the riparian vegetation community structure, and hence the allochthonous input. *Acacia mearnsii* (Black wattle) and *Nematolepis squamea* (Lancewood) grow more readily in the upper catchment but are rarely present along streams occurring in the basalt soils. The surface structure and texture of siltstone rocks is faceted and smooth, but also more easily inclined to fracture, compared with basalt rocks which slowly and unevenly weather, creating a porous appearance, thus basalt may be more stable and more readily provide sheltering sites for snails. Field observations of rock surfaces also suggests that the rate of periphyton growth is slower on siltstone (K. Richards personal observation). Mineral accessibility, surface roughness, periphyton growth and riparian contributions combined are likely to explain the distribution of *Beddomeia* in relation to geology at Castra.

Catchment size and disturbance were identified as the most influential explanatory factors for the Groom River dataset. Despite high levels of anthropogenic disturbance in both river catchments, with more recent and widespread agricultural and forestry disturbances occurring within the Castra Rivulet catchment, disturbance was only identified as a significant factor in the models explaining snail abundance at Groom River. Evidence of historic mining disturbance was present in, or adjacent to, most of the study streams in the Groom River catchment, showing signs of significant channel modifications. Although natural regeneration has been allowed to occur throughout much of the catchment for approximately 90 years, there can be no certainty about the severity of the environmental impacts, caused by tin mining, on individual streams. Interestingly, despite this intensive stream channel disturbance, *Beddomeia* spp. still occur in most streams, indicating that populations can recover after time following high level disturbances.

While natural dispersal mechanisms such as downstream transport on CPOM may be responsible for the widespread occurrence of *Beddomeia* spp. throughout Groom River, historic records show that alluvial tin mining practices did greatly impact upon the catchment causing severe degradation of many stream channels. Mining was also responsible for removal of forest cover over wide sections of the catchment, to supply timber for the water-races and settlement buildings (Lesser 1987, Jackman 1998). Such disturbances are likely to have resulted in extinction of some populations within more impacted streams, for example, those associated with the Anchor Creek mining operation. Conversely, mining operations may also have been the mechanism for translocation of *Beddomeia* spp. to certain sites. It is probable that reintroduction of snails to highly modified or eroded streams could have resulted from modifications to the catchment hydrology, through the formation and operation of several large-scale systems of water races across the catchment to support the mining (Department of Mining data presented in Jackman 1998). These races operated for approximately 50 – 70 years, but have subsequently since been decommissioned, lapsing into a state of disrepair and no longer carry water; just how recently individual sections ceased functioning is unknown.

3.5.3 Population structure

A number of age cohorts were recognised in this study, the relative proportions of which were similar across the Groom River headwater streams but somewhat more variable at Castra. While it is probable that the number of cohorts identified may be an overestimate, being derived from continuous data, the information may be useful in assessing the relative health of snail populations in headwater streams (although perhaps less effective for within-stream applications or for larger streams possessing few snails). In most of the headwater streams in this study, *Beddomeia* populations were relatively stable (combined site data), containing all six cohorts in similar relative proportions in each stream, however more variation occurred in the Castra streams. For example there is some evidence to suggest a disturbance event at Ca5 in recent times as based on population structure. In this stream, juvenile, S4 and S3 cohorts contributed only 20% to the population indicating a stall in reproduction in the recent past, possibly around the time of harvesting of the adjacent forest two years prior to initiating this study. Greater variability in population structure was apparent in higher order streams, resulting from small population sizes. The use of population structure to assess population health in larger streams is thus limited by low snail abundances influencing the interpretation of data.

3.5.4 Morphotypes

Previous studies have reported sympatric distributions amongst hydrobiid species (e.g. Ponder *et al.* 1994). In the current study, several sympatrically occurring morphotypes of *Beddomeia* were detected in most streams of the two river catchments, while *Austropyrgus* spp. also co-occurred with *Beddomeia* at Castra. Snail presence and abundance was shown to vary between streams and sites on streams, in each catchment but a single morphotype was usually more abundant, as was the case at 90% of sites and > 75% of the streams. The maximum number of morphotypes per stream fluctuated, but the abundance of several were too low or too variable to offer any valuable insight into behaviour or habitat preferences. Descriptions of the morphotypes are presented in Chapter 5 and their molecular genetics explored in Chapter 6 to determine whether sufficient anatomical and molecular differentiation exists to support the number of morphotypes recognised in each catchment.

3.5.5 Conservation management implications

This research constitutes the first spatial and temporal catchment-wide study on *Beddomeia* spp. in Tasmania and contributes to our understanding of the ecology of headwater stream hydrobiids. A number of key findings have been identified from this research which have implications for the management of *Beddomeia* species:

This research has shown that the *Beddomeia* spp. investigated are headwater stream specialists and are not found in high numbers in higher order streams. Whereas once they were thought to be restricted to single sites or small catchments (Ponder *et al.* 1993), this study has demonstrated that some species of *Beddomeia* at least occupy multiple streams and have a wider catchment distribution. This study has also increased our understanding of spatial and temporal distribution of *Beddomeia* spp. and introduces the concept of variability in population densities between superficially similar streams, the implications of which are that other *Beddomeia* species may also display similar behaviours and have broader distributions than currently known.

The results suggest that some previously held concerns about the effects of anthropogenic disturbance on *Beddomeia* populations may be unwarranted, including that recovery from such disturbances can occur, although we cannot discount that local population extinctions may also have occurred. Indeed the effects are not straight forward and may be species-

specific, suggesting further exploration is needed to build a clearer picture of the impacts of disturbance.

Finally, multiple morphotypes were recorded, often living sympatrically, in each study catchment, and the pattern of distribution of some threatened species, *B. tasmanica* (morphotypes 1, 2 and 6) and *B. hallae* (morphotype B) identified in the catchments were more widespread than previously suspected (refer to chapter 5 and 6). However, distribution was patchy and abundances varied between streams, and the data was unable to fully explain why some streams failed to support snail populations.

One objective of this ecological study was to determine whether it is possible to predict high quality habitat based on the habitat parameters identified. The best regression models produced were able to explain about 60% of the variation within the datasets, which is a reasonable model for ecological data. In each case the models were able to identify a subset of important explanatory habitat parameters; however, as a management tool these models are not likely to be of much benefit, as many of the explanatory variables identified are insufficiently detailed to detect differences between some streams containing snails and others without snails, particularly in the Groom River catchment. Additionally, as this research shows, species within the two study catchments respond to different sets of habitat variables, and live in different habitats to large-river inhabiting *Beddomeia* species, restricting the usefulness of the models produced.

3.6 Conclusion

This research has contributed to our understanding of stream snails in Tasmania, and has demonstrated the importance of headwater streams to *Beddomeia* spp. in both catchments. Areas of future research have been highlighted which may offer improvements in predicture modelling, such as the need to understand the influence of geology on snail distribution, particularly on other species and where other geological transitions occur. Such findings have implications for future management of *Beddomeia* species, which will be explored in Chapter 7.

3.7 Appendix

Appendix A. Site locations (in GDA) and snail abundance per sub-site. Shaded areas indicate no sample was taken.

Site	Easting	Northing	*Beddomeia* abundance per sub-site			
			A	B	C	D
1ACa1	422492	5423173	0	0		
2ACa1	422352	5423083	0	0		
3ACa1	422122	5423063	0	0		
1ACa2	422872	5423143	0	0		
2ACa2	422832	5423183	0	0		
3ACa2	422752	5423223	0	0		
1ACa3	422892	5422983	0	0		
2ACa3	422872	5422883	0	0		
3ACa3	422832	5422783	0	0		
1ACa4	424292	5422893	148	370	115	175
2ACa4	424132	5422963	18	33	45	13
3ACa4	424032	5423003	33	7	16	304
1ACa5	425972	5422383	17	7	17	3
2ACa5	425912	5422433	1	0	1	1
3ACa5	425812	5422533	14	0	0	0
1ACa6	426572	5422143	32	119	71	258
2ACa6	426682	5422103	72	280	44	170
3ACa6	426882	5421963	14	156	41	130
1ACa7	427122	5422683	41	211	163	258
2ACa7	427232	5422733	296	580	111	665
3ACa7	427352	5422663	110	287	357	621
2ACa8	427432	5422943	10	7	4	18
3ACa8	427432	5422983	20	6	13	21
1ACa9	427432	5422773	10	22	11	13
2ACa9	427432	5422833	9	6	5	4
3ACa9	427432	5422893	9	13	7	4
1ACa10	424592	5422323	32	115	56	252
2ACa10	424592	5422263	83	143	111	325
3ACa10	424592	5422193	36	89	150	273
1ACa11	424512	5422123	36	54	23	59
2ACa11	424472	5422063	20	52		0
1ACa12	424552	5422363	0	3	0	0
2ACa12	424472	5422443	0	2	0	0
3ACa12	424352	5422453	0	0	2	1
1ACa13	423192	5421443	0	0		
2ACa13	423072	5421503	0	0		
3ACa13	422992	5421523	0	0		
1ACa14	423412	5421983	0	0	0	0
2ACa14	423312	5422083	0	0	0	0
3ACa14	423252	5422183	1	0	0	
1ACa15	423382	5421913	1	0	4	5
2ACa15	423312	5421923	0	0	0	3
3ACa15	423212	5421933	0	0	0	0
1ACa16	423552	5421883	1	5	5	9
2ACa16	423432	5421923	0	4	2	10
3ACa16	423482	5421903	2	24	16	31
1ACa17	424652	5421323	301	289	198	291
2ACa17	424682	5421483	104	154	101	156
3ACa17	424672	5421683	132	438	108	78
1ACa18	424872	5421163	6	10	0	20
2ACa18	424752	5421183	1	7	3	2
3ACa18	424642	5421303	3	12	142	8

Site	Easting	Northing	*Beddomeia* abundance per sub-site			
			A	B	C	D
1AGC1	581172	5436363	721	226	559	444
2AGC1	581062	5436333	296	286	117	242
3AGC1	580952	5436303	295	147	140	
1AGC2	581542	5436993	19	42	105	17
2AGC2	581462	5437023	63	16	5	20
3AGC2	581412	5437093	24	20	13	3
1AGC3	581572	5437023	13	4	6	2
2AGC3	581612	5437103	3	2	0	4
3AGC3	581672	5437183	10	14	6	6
1AGC4	583692	5436703	0	0		
2AGC4	583642	5436733	0	0		
3AGC4	583562	5436783	0	0		
1AGC5	584232	5436233	0	0	0	0
2AGC5	584342	5436353	8	0	0	1
3AGC5	584362	5436593	0	0	0	0
1AGC6	584932	5434983	0	0		
2AGC6	584892	5435103	0	0		
3AGC6	584872	5434963	1	0		
1AGC7	585547	5435103	267	105	37	367
2AGC7	585632	5435183	667	618	233	651
3AGC7	585712	5435233	505	106	35	69
1AGC8	585492	5435118	22	39	23	31
2AGC8	585512	5435223	319	194	194	79
3AGC8	585607	5435383	154	99	99	246
1AGC9	585392	5435553	0	0	0	0
2AGC9	585472	5435633	19	44	31	66
3AGC9	585552	5435733	37	64	233	99
1AGC10	585312	5434983	31	9	11	6
2AGC10	585372	5435043	14	1	6	5
3AGC10	585442	5435063	15	9	17	21
1AGC11	585762	5433403	0	1	0	0
2AGC11	585662	5433363	0	1	0	0
3AGC11	585602	5433303	0	0	0	12
1AGC12	586362	5433583	13	6	4	12
2AGC12	586462	5433633	52	8	14	16
3AGC12	586592	5433783	34	7	6	156
1AGC13	587282	5433223	2	3	0	2
2AGC13	587352	5433303	5	0	0	0
3AGC13	587252	5433263	1	23	12	1
1AGC14	586762	5432278	0	1	0	0
2AGC14	586707	5432253	2	2	0	0
3AGC14	586672	5432303	3	0	1	1
1AGC15	586032	5431453	405	227	431	651
2AGC15	586002	5431313	1369	318	883	734
3AGC15	586002	5431223	291	28	85	
1AGC16	586602	5432363	46	23	63	44
2AGC16	586462	5432423	0	0	0	0
3AGC16	586332	5432363	27	6	8	4
1AGC17	584962	5431993	21	22	1	50
2AGC17	584852	5431963	72	7	14	52
3AGC17	584712	5431943	1447	675	601	243
1AGC18	584562	5433023	14	24	12	12
2AGC18	584517	5432953	48	12	24	30
3AGC18	584502	5432883	155	385	300	664

Appendix B. Key to Degree of disturbance table (Table 3.3)

The degree of disturbance to each study stream catchment was determined based on the following information.

Disturbance grade

Is a subjective assessment of disturbance based on percentage of catchment disturbance (from nil to 100% above site, presence of riparian zone and forest (from 100% to 0)), on a scale 0-5.

Catchment disturbance (%)	Forest and SSR quality	Disturbance category
Nil	Riparian trees and forest continuous	0
Historic selective harvesting	Riparian trees and forest continuous	1
Partial clearing (70% remaining, understorey vegetation present)	Riparian trees and forest continuous	1.5
Partial clearing (70% remaining)	Some stream vegetation damage	2
50% cleared	Riparian trees discontinuous, high degree of forest removal	3
80% cleared, burnt	Riparian trees present	4
80% cleared, burnt	Riparian trees absent	4.5
100% cleared	cleared	5

*SSR = streamside reserve, or riparian buffer

Categories used in Degree of disturbance column:

1. *Forestry operations within the study stream catchment*
    a. logging – recent (within past year) – clearfall (CLF) to *Pinus radiata* plantation (PP)
    b. recent (within past 2 years) – CLF to PP
    c. recent (within past 5 years) – CLF to PP
    d. recent (within past year) – CLF to *Eucalyptus nitens* plantation
    e. recent (within past 2 years) CLF to *E. nitens* plantation
    f. recent (within past 5 years) CLF to *E. nitens* plantation
    g. recent (within past 1, 2 or 5 years) CLF to regeneration
    h. previously harvested (within 30 years) to PP, or regenerating native forest

2. *Mining*
    a. historic - total forest and stream destruction, regenerating
    b. historic - use of waterways only (detour water via water races)
    c. historic - remaining archaeological sites

3. *Agriculture*
    a. Clearfall (CLF) to pasture
    b. CLF to pasture with some streamside buffers (SSRs) – introduced weeds
    c. CLF to pasture with appropriate forestry SSRs retained

4. *Roading*
    a. forestry access road across SSR
    b. main road across SSR
    c. forestry snig track near SSR

5. *Public access/walking tracks*
    a. heavily trodden (impacted) across stream, between or above sites
    b. heavily trodden (impacted) adjacent to stream
    c. evidence of continuous/intermittent/infrequent public access

6. *Weed infestation*

Appendix C. Parameter estimate tables for multiple regression of catchment data by habitat type.

The row labelled "(Intercept)" in each table represents the log abundance of snails in *Eucalyptus obliqua* forest (fcO10) when all the continuous variables take the value zero (applies to Tables A – F).

Table A. Parameter estimates for Groom River rock habitat data.

	Estimate	**SE**	***t* value**	**Pr(>|*t*|)**
(Intercept)	5.80338	0.61719	9.403	< 2e-16 ***
Riparian.slope.1	0.03923	0.01233	3.183	0.001768 **
Catchment	-0.64890	0.09364	-6.930	1.10e-10 ***
Disturbance.grade	-1.12918	0.15753	-7.168	3.02e-11 ***
fcRC1.2	0.08639	0.44826	0.193	0.847425
fcSD6	-0.15114	0.60965	-0.248	0.804531
fcSE1	5.35223	0.66264	8.077	1.84e-13 ***
fcSE1 – 2	5.85355	1.56845	3.732	0.000267 ***
fcSE1 – 3	4.43277	1.56845	2.826	0.005340 **
fcSE1 – 4	4.24202	1.56845	2.705	0.007614 **
fcWR101	1.29413	0.34513	3.750	0.000251 ***
fcWR110	0.62565	0.45014	1.390	0.166579

Residual standard error: 1.491 on 153 degrees of freedom

Table B. Parameter estimates for Groom River CPOM habitat data.

	Estimate	**SE**	***t* value**	**Pr(>|*t*|)**
(Intercept)	8.391446	0.821668	10.213	< 2e-16 ***
Aspect	-0.008182	0.002057	-3.977	9.98e-05 ***
Catchment	-0.943439	0.115303	-8.182 4	28e-14 ***
disturbance.grade	-1.012659	0.201780	-5.019 1	21e-06 ***
fcRC1.2	0.048216	0.551550	0.087	0.930433
fcSD6	1.700991	0.702993	2.420	0.016498 *
fcSE1	3.256810	0.909149	3.582	0.000435 ***
fcSE1 – 2	-0.716502	2.093527	-0.342	0.732552
fcSE1 – 3	2.004369	2.093527	0.957	0.339602
fcSE1 – 4	2.572140	2.093527	1.229	0.220768
fcWR101	2.001636	0.409917	4.883	2.24e-06 ***
fcWR110	0.888987	0.565298	1.573	0.117512

Residual standard error: 2.002 on 186 degrees of freedom

Table C. Parameter estimates for Groom River substrate habitat data.

	Estimate	**SE**	***t* value**	**Pr(>|*t*|)**
(Intercept)	-0.66375	0.32217	-2.060	0.04083 *
Catchment	-0.36857	0.08065	-4.570 9	09e-06 ***
fcRC1.2	0.92330	0.31524	2.929	0.00385 **
fcSD6	2.11624	0.52239	4.051	7.60e-05 ***
fcSE1	2.66274	0.57786	4.608	7.73e-06 ***
fcSE1 – 2	2.76264	1.50624	1.834	0.06830 .
fcSE1 – 3	4.39156	1.50624	2.916	0.00401 **
fcWR101	0.55569	0.31274	1.777	0.07730 .
fcWR110	1.56017	0.36607	4.262	3.28e-05 ***

Residual standard error: 1.481 on 178 degrees of freedom

Table D. Parameter estimates for Castra rock habitat data.

	Estimate	**SE**	***t* value**	**Pr(>|*t*|)**
(Intercept)	9.56596	1.73446	5.515	1.40e-07 ***
Catchment	-0.62300	0.10903	-5.714	5.39e-08 ***
Geology O*	-2.56641	0.48887	-5.250	4.88e-07 ***
Geology S	-2.49422	0.51998	-4.797	3.72e-06 ***
fcPPR	-0.21012	0.76673	-0.274	0.78441
fcRC1.1	-0.70777	0.45091	-1.570	0.11851
fcRC1.2	1.10703	0.74541	1.485	0.13951
fcWO0110	-0.88675	0.56559	-1.568	0.11893
fcWR101	0.11551	0.58094	0.199	0.84265
Altitude	-0.01191	0.00382	-3.118	0.00216 **
Flow	-2.71630	0.97075	-2.798	0.00578 **

Residual standard error: 1.64 on 157 degrees of freedom

*Geology O refers to the transitional zone betweengGeology B (basalt) and geology S (siltstone) and contains rocks of both types.

Table E. Parameter estimates for Castra CPOM habitat data.

	Estimate	**SE**	**t value**	**Pr(>|t|)**
(Intercept)	4.98032	0.53067	9.385	< 2e-16 ***
Catchment	-0.89683	0.09723	-9.224	< 2e-16 ***
Geology O	-4.75308	0.37989	-12.512	< 2e-16 ***
Geology S	-4.06676	0.34560	-11.767	< 2e-16 ***
fcPPR	0.26340	0.64080	0.411	0.681547
fcRC1.1	0.65967	0.39375	1.675	0.095692 .
fcRC1.2	2.80993	0.74485	3.772	0.000223 ***
fcWO0110	-0.30184	0.52044	-0.580	0.562694
fcWR101	1.67733	0.47913	3.501	0.000592 ***

Residual standard error: 1.705 on 171 degrees of freedom

*Geology O refers to the transitional zone betweengGeology B (basalt) and geology S (siltstone) and contains rocks of both types.

Table F. Parameter estimates for Castra substrate habitat data

	Estimate	**SE**	***t* value**	**Pr(>|*t*|)**
(Intercept)	1.4406	0.2758	5.224	5.39e-07 ***
Catchment	-0.4472	0.0730	-6.126	6.75e-09 ***
Geology O	-2.2864	0.2893	-7.903	4.15e-13 ***
Geology S	-2.1780	0.2524	-8.628	5.88e-15 ***

Residual standard error: 1.346 on 160 degrees of freedom

*Geology O refers to the transitional zone betweengGeology B (basalt) and geology S (siltstone) and contains rocks of both types.

Chapter 4

An Investigation of the Impacts of Cable-harvesting on *Beddomeia* species

Although several studies have examined the effectiveness of riparian buffers on stream macroinvertebrate communities in the production forests, some current harvesting methods by their nature do not offer protection of stream buffers on headwater streams. Chapter 4 investigates the impacts of one such method of forest harvesting, cable-harvesting, on a population of hydrobiids. Such studies are useful in determining important habitat and where conservation measures would best be focused in order to improve conservation outcomes.

Headwater stream following forest harvest and regeneration burn

4 Impacts of native forest cable-harvesting on a population *Beddomeia* spp. (Hydrobiidae: Mollusca) in NE Tasmania

4.1 Introduction

Land use changes such as forest harvesting and agricultural clearing have the potential to severely degrade stream environments, affecting water quality and the ecology of the aquatic community (Bunce *et al.* 2001, Cornish 2001, Bramley and Roth 2002, Cascorbi 2002). Overstorey removal and increased surface flows impact upon stream channel morphology and wood recruitment (Bunce *et al.* 2001, Dahlstrom and Nilsson 2004, Davies *et al.* 2005a, Davies *et al.* 2005b, Meleason and Hall 2005), while in the case of forestry operations, post-harvest regeneration burns and sediment runoff resulting from roading have been observed to change water chemistry, both under Australian native eucalypt forest types (e.g. Campbell and Doeg 1989) and coniferous forests in Washington State, U.S.A., and Canada (e.g. Kreutzweiser and Capell 2001, Rashin *et al.* 2006). The extent of these impacts is dependent on the type and scale of the operation and the cumulative effects of multiple operations within the catchment.

Forest managers often apply protective measures such as riparian buffer strips (streamside reserves) to ameliorate impacts of forestry operations, but their efficacy is dependent on the stream size, the width of the buffer strips, geology and forest type (Davies and Nelson 1994, Jones *et al.* 1999, Boothroyd *et al.* 2004, McIntosh and Laffan 2005). The demonstrated effects of timber harvesting operations on macroinvertebrate communities include changes in the composition and abundance of functional feeding groups, and a shift from carnivore dominated communities to ones representative of slower flowing habitats, dominated by oligochaetes, dipterans and nematodes (Growns and Davis 1991, Davies *et al.* 2005a). However, changes in macroinvertebrate community composition are not universal; Watson (2004) found considerable variation in functional group composition between both logged and unlogged streams. Despite this, the overall response of macroinvertebrates to riparian management appear similar for boreal, sub-boreal and eucalypt-dominated forests. However, limited information is available on the impacts of forest harvesting on freshwater molluscs.

The effectiveness of buffer strips in protecting stream biodiversity and in-stream habitats is reliant on their width and structural integrity (e.g. Danehy *et al.* 2007, Thompson *et al.* 2009);

widths of riparian buffer strips applied vary between and within countries (McDermott *et al.* 2007). Buffer strips of between 0 – 10 m wide on streams with catchments of < 50 ha have been shown to be ineffective in maintaining macroinvertebrate community composition (Davies and Nelson 1994, Davies *et al.* 2005a) as a result of increased sedimentation and altered channel morphology. Inadequate buffer widths may also affect the level of invertebrate drift in headwater streams (Hoover *et al.* 2007). It is generally accepted in Australia that buffers of between 10 - 30 m minimise changes to community composition, streams with smaller catchment sizes having smaller buffers (Davies and Nelson 1994, Smith *et al.* 2009), although on sandy substrates in W.A. changes to the composition of the macroinvertebrate community have still been recorded despite buffers of between 50 to 100 m (Growns and Davis 1994). The extent and effects of riparian disturbance on aquatic vertebrates has also been tested and varies with the size of buffer widths. For example, there is evidence that for some fish species in heavily forested watersheds in the U.S.A., cumulative effects of harvesting in upstream catchments, including disruption to riparian-zone trees over more than 1 km in length of stream, is not tolerated by the species (Jones *et al.* 1999). The responses of periphyton to experimental manipulation of buffers also indicates an observable photosynthetic difference can be measured between controls and buffered streams, even those containing 30 m buffers (Kiffney *et al.* 2003). The structural integrity of buffers may be impaired by tree death as a result of windthrow or burning, through floristic changes triggered by altered microclimates, or by approved management activities allowing timber removal. Any of these factors may compromise the effectiveness of buffers to limit impacts on stream ecology and functioning (Forest Practices Board 2000a, Dignan and Bren 2003, McDermott *et al.* 2007), as may the level of frequency, magnitude and duration of these environmental stressors.

Compositional changes to macroinvertebrate communities, amounts of allochthonous material, and alteration to channel morphology and substrate structure in headwater streams have been shown to remain measurable up to 15 years after harvesting, both in situations where riparian buffers were prescribed and also where no buffers where retained (Growns and Davis 1991, Young *et al.* 1994, Davies *et al.* 2005a, Davies *et al.* 2005b). Likewise, the persistence of coarse in-stream woody debris (CPOM) resulting from harvesting has been reported in streams both with and without riparian buffers, showing an initial increase in CPOM that subsequently reduces with time (Dahlstrom and Nilsson 2004, Davies *et al.* 2005b). Increased CPOM has been recorded to be retained in Alaskan streams for as long as 37 years after harvest, although total amounts decline with time (Gomi *et al.* 2001). The initial increase in available woody material post-harvest is likely to explain observed

increases in invertebrate biomass at ‚recently logged' study sites in sub-boreal forests in British Columbia (Fuchs *et al.* 2003); however, the longevity and thus the usefulness of this resource is likely to differ depending on the tree species.

Many species of Hydrobiidae (Mollusca) and related families, are small stream specialists, being most abundant in streams with low flows and debris accumulation, and also in seepages containing filamentous algal growth (e.g. Miller *et al.* 1999, Sada 2001, Clark *et al.* 2003, Sada 2008; this thesis Chapter 3). *Beddomeia* is a hydrobiid genus endemic to northern Tasmania, species of which occupy streams across a range of different catchment sizes (Ponder *et al.* 1993). Thirty-seven of the 46 described species of *Beddomeia* are currently listed as rare, vulnerable or endangered on the Tasmanian *Threatened Species Protection Act, 1995*, due to their restricted distributions and on-going threatening processes; the habitat for most of the listed *Beddomeia* species occurs within production forest or areas disturbed by agricultural practices.

Management of species within the genus *Beddomeia* is currently hampered by insufficient knowledge of their habitat preferences and response to habitat disturbance. Like many species of Hydrobiidae, *Beddomeia* species have not, until recently, been well studied due in part to their size and their cryptic habits, living as they do in gravel or on the underside of rocks, leaves and logs in streams (Ponder *et al.* 1993; this thesis Chapter 1, 3). What little is known about Australian native hydrobiid reproduction and behaviour suggests they have extremely limited dispersal capabilities, with many species known only from single localities, individual mound springs, or small catchments (Ponder *et al.* 1993, Ponder *et al.* 1994, Ponder *et al.* 1995, Davies and Cook 2002, Clark *et al.* 2003, Spiers 2003). Recent work investigating the catchment-wide spatial and temporal distribution of *Beddomeia* spp. highlights the importance of headwater streams to the ecology of some *Beddomeia* species (Chapter 3).

It has previously been reported that disturbances brought about by human-induced land use changes including agriculture, dam construction, mining and forestry are likely to have detrimental consequences for freshwater molluscs, including *Beddomeia* spp. (Ponder *et al.* 1993, Ponder 1997b, Ponder and Walker 2003, Strong *et al.* 2008). This study aims to determine whether one method of forest harvesting (cable-harvesting), which by its very nature results in the severe modification of the riparian zone of headwater streams, has an effect on populations of *Beddomeia*, and, if so, to determine where conservation measures would best be focused. To achieve these aims, a retrospective study investigating the impacts

of a cable-harvesting operation on a high-density population of a *Beddomeia* species was conducted in northeast Tasmania between 2002 and 2007. Aspects of population abundance and structure were investigated and compared with a control stream population to determine what effects, if any, the harvesting had on the population, to ascertain the factors responsible for the observed changes and to identify signs of recovery.

4.2 Methods

4.2.1 Study design and location

The study was conducted in two streams in the Goulds Country State Forest, northeast Tasmania (Figure 4.1). Geology (dominantly adamellite/granite and associated dykes or alkali-feldspar granite), aspect (westerly), forest type (mature wet *Eucalyptus regnans* and *E. obliqua* forest) and physicochemical nature of the sites (e.g. pH, electrical conductivity, dissolved oxygen, turbidity and water temperature) were consistent across the treatment and control streams.

Treatment sites were established in a 39 ha patch of regenerating native forest that had been logged and burnt in 1999/2000 using the cable harvesting method (Forest Practices Board 2000a). The headwaters of the treatment stream were in mature eucalypt forest 140 m upstream of the upper boundary of the forest operation and the harvest area contained a length of disturbed channel (600 m), downstream of which the stream continued through mature forest. The control stream was located in unharvested native forest, approximately 1.5 km to the north-west (Figure 4.1). There were no other forms of land-use disturbance in either catchment.

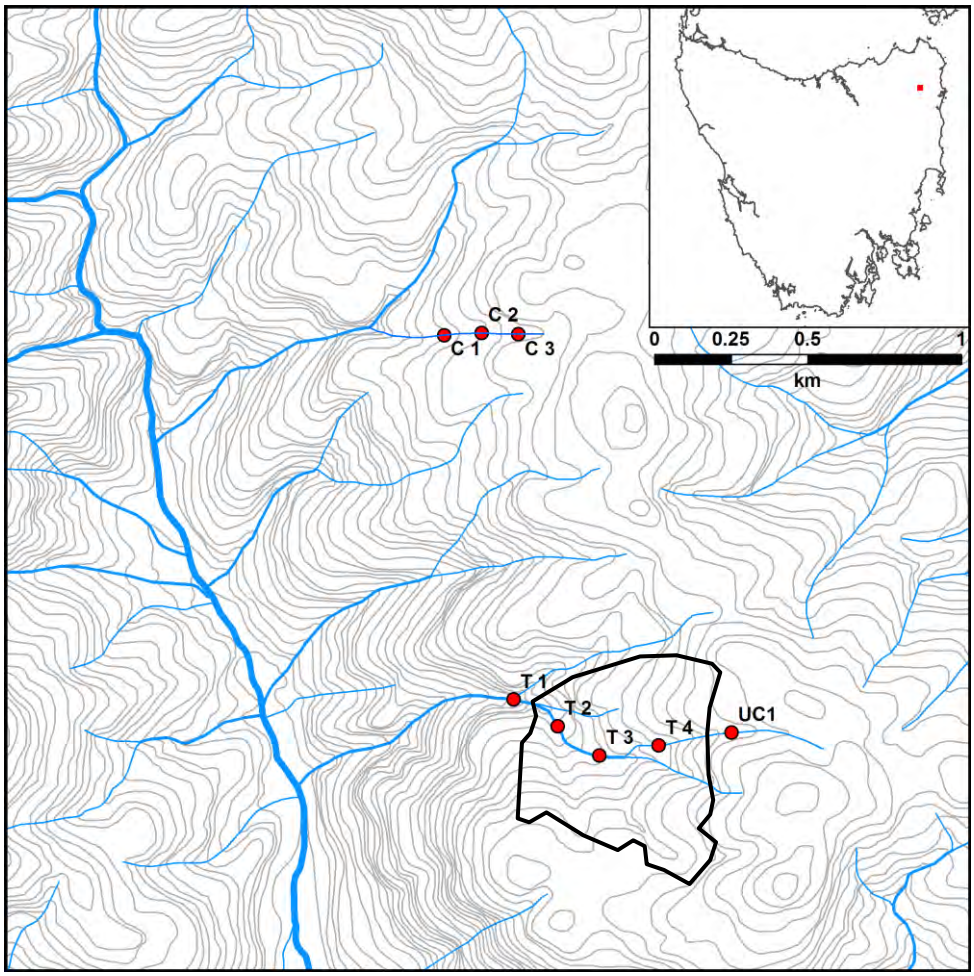


Figure 4.1. Location of study sites. T1 – T4 are treatment sites, UC1 the upstream control and C1 – C3 are sites on the control stream. Harvest area is represented by the black outline.

The treatment area incorporated a first order stream flowing through the harvested area, supplemented by a small ephemeral feeder tributary originating within the operation area and joining the main channel below site 2; the second feeder tributary above T3 did not carry surface water (Figure 4.1). Five sites were established along the main branch of the headwater stream; the location of sites is illustrated in Figure 4.1 and grid references of sites are presented in Table 4.1. Three sites, (T2 – T4) were positioned along the stream within the operation area, one site (T1) was situated in unharvested forest, 100 m downstream of the operation, and one site, (UC1) used as a pseudo-control, was located in mature forest 50 m immediately upstream of the operation. A further three control sites (C1 - C3) were established on a nearby stream (Figure 4.1). Sites were separated by a stream length of 150 m and each site was divided into sub-sites of 5 m lengths (A – E).

Catchment size upstream of each site is presented in Table 4.1. The catchments of treatment sites T1, T2 and T3 were similar in size to the catchments of control stream sites C1, C2 and C3.

Table 4.1. Location of site (in GDA) and catchment size above each site.

Site	Grid Reference		Catchment (ha)
	Easting	Northing	
T1	582834	5432255	35.6
T2	582959	5432176	25.3
T3	583115	5432144	12.8
T4	583306	5432126	7
UC1	583541	5432193	4.2
C1	582610	5433410	15.3
C2	582730	5433440	10.9
C3	582850	5433450	6.9

* based on GIS Tasmap (Zone55) 1:25000 scale topographic map

4.2.2 Fauna sampling

Sampling of the treatment stream was conducted annually in spring from 2002 to 2007, with the exception of 2005, when weather conditions prevented it. Sites on the control stream were established in 2006 and sampled in 2006 and 2007.

An ordered sampling design was employed to sample sub-sites, with sub-site A (most downstream at each site) sampled in the first sampling season, through to sub-site E (uppermost) in 2007. This approach was used to reduce the possible impacts that a random design of sampling might have had on the natural habitat availability (amount of natural CPOM in-stream) and to avoid any effects of alteration to sedimentation caused by upstream sampling events. Three habitat types: rocks, allochthonous material or coarse particulate organic matter (CPOM), and substrate were sampled at each sub-site, where available, for the first two sampling events, using a modified „washing method' (Chapter 2); following which only rocks and CPOM were sampled. Habitat parameters at each site were recorded at each visit and the presence of *Beddomeia* egg capsules, where visible on the habitat type, was noted.

A semi-quantitative sampling methodology was applied to the study as used in Chapter 3. Rock, CPOM and substrate sampling time was restricted to 20 minutes per habitat type and

sampling procedures were standardised: the volume of CPOM collected per sub-site was limited to one 36 x 26 x 5cm sampling tray, the number and proportions of rocks sampled included 25 pebbles (< 6 cm diameter) + 25 cobbles (> 6 < 25 cm) + 2 boulders (>25 cm) where available; and 5 x 200 ml of gravel substrate was collected. Samples were obtained by the „washing method' (Chapter 2), and rock and CPOM habitats were sampled separately. Habitat material was washed in a bucket of water, the habitat surfaces agitated by hand to remove molluscs. The bucket contents were then sieved through a 300 µm mesh net and the contents transferred into 200 ml containers and preserved in 5% formalin, or 70 % ethanol. The stream substrate was sampled by removing five 200 ml jars of stream bed substrate from an area of 50 x 50 cm of stream bed in slower flowing sections of the sub-site. Each container was lowered, mouth-side down, onto the substrate and material scooped into the container from beneath, whilst simultaneously placing a 300 µm mesh net immediately downstream to catch any disturbed substrate. Container and net contents were then sieved through another 300 µm mesh net and the contents transferred into a 500 ml container and preserved in 70% ethanol.

Samples were processed in the laboratory with the aid of a dissecting microscope (Leica MZ75). Aquatic molluscs were identified to genus and counted, while *Beddomeia* were further classified into morphotype, based on shell characters (Chapter 3, 5), and counted. Morphotypes were also categorized into size class, based on the number of body whorls, to obtain population structure data. The age structure of the *Beddomeia* population was recorded for all sites at each sampling event. Snail abundances, densities (no. cm^{-2} and no. per tray CPOM) and population age structure were obtained for each substrate type at each site. The presence of *Beddomeia* spp. egg capsules on material collected was also recorded and micro-habitat type (CPOM, rock or gravel substrate) noted.

4.2.3 Population data

Population structure was obtained by classifying snails into size categories. Adult size (shell height in mm) varied between the four morphotypes recorded in this study, therefore, in order to obtain accurate population age structure the snails were first sorted into morphotypes and then classified by size (based on the number of whorls and calcification of outer lip signifying mature adults); six size classes were detected, i.e. Adult, S4, S3, S2, S1 and Juvenile. Due to the low abundance of some morphotypes, the data were combined to give a single population structure.

4.2.4 Habitat variables

The parameters recorded at each site used in the final analyses are listed in Table 4.2. Average channel depth was measured at four random locations in the flowing section of channel within each sub-site. Likewise, average width was calculated from four measurements including maximum and minimum widths within each 5 m sub-site.

Table 4.2. List of habitat variables used in the final analyses.

Variable	Description
Slope	Degrees of the slope of the sub-site. (using clinometer)
Year	Sampling event (1 to 5)
Stream	Treatment or control stream (no. 1 and 2 respectively)
Stream order	Classification of stream based on order. (Strahler 1957)
Catchment size	Measure of catchment size above the site (log transformed).
Riparian slopes	An estimate in degrees of the slope of the riparian zones immediately adjacent to each sub-site (slope 1 and 2 for LHS and RHS respectively facing upstream).
Substrate composition	A measure allocated to categories of the substrate composition (%) at each sub-site. (Categories include: boulders, cobbles, pebbles, gravel, sand and mud (or other)) Only boulders, cobbles and pebbles are used.
Cover of CPOM in-stream	Cover by woody debris (CPOM, in percentage) of in-stream substrate.
Depth (average)	Average depth (cm) from 4 records across the sub-site.
Width (average)	A measure of the average width (cm) of the sub-site. (5 m of stream)
Stream composition	A measure of the composition of the stream (%) in each sub-site. *(% pool, riffle, run*
Average stream flow (m/s)	Average flow (m/s) recorded at each sub-site.*(calculation from average of 4 measurements – flow 1, 2, 3, 4, in m/s)*
Width	Average width of sub-site in cm (average of 4 measurements)
Depth	Average depth of sub-site in cm (average of 4 measurements)
Flow	Flow of stream at sub-site in $m.s^{-1}$(average of 4 measurements)
Forest age	Age, in years, of surrounding forest cover. (Arcsine square-root transformed owing to preponderance of high and low values)
Rocks washed	A count of the number of rocks washed (and therefore no. present). *(number of boulders (max of 2), cobbles (max 25), pebbles (max 25) washed)*
Tray volume	A measure of the volume of CPOM sampled. (fraction of tray)

4.2.5 Analyses

Snail abundance and population structure between sites on the treatment stream

Data were analysed using an unreplicated two-way ANOVA with site and year being treated as ordered, fixed factors; treating the factors as ordered permitted the use of orthogonal

polynomial contrasts was used to test for linear, quadratic and higher order trends across sites or years. Inspection of the data suggested that including the data from site T3 in the analysis would clearly violate the assumption of additivity (Quinn and Keough 2002), and so the formal analysis omitted this site. There is some chance that the data are autocorrelated over time, although formal testing of this assumption is impossible owing to the short time sequence available. Accordingly, the *p*-values may overestimate the significance of any difference between sites or years. Inspection of diagnostic plots suggested a square-root transformation would be adequate to meet the other assumptions of ANOVA ($p < 0.0001$), and this was the case for these analyses.

Snail abundance and population structure between the control sites

A two-way unreplicated ANOVA was used to test for linear, quadratic and higher order trends across sites or years, although there were only two years where observations were taken in all the control sites. The principal interest was in comparing the other control sites with the „upstream' control in the cable harvest stream. Square root transformation was again appropriate to meet the assumptions of ANOVA, and preliminary plots showed no violations of the assumption of additivity.

Relationship between habitat variables and snail abundance

Step-wise regression analyses using JMP version 4.0.0 (SAS Institute Inc. 1989-2000) were conducted using site as a random factor. Some of the habitat variables were log transformed to improve normality: catchment size, canopy cover and *Beddomeia* abundance. Several measures of snail abundance (i.e. total abundance, log-transformed abundance, density no. cm^{-2} rock surface and density per tray of CPOM) were regressed against the environmental data to test for the most appropriate measure to use as the response variable and to identify habitat variables potentially influencing snail abundance. The criteria for including variables in models was $P < 0.05$. The final regression analyses were conducted on the total *Beddomeia* on CPOM data and rocks datasets independently, due to the differences in sampling and data handling.

4.3 Results

4.3.1 Snail abundance

A total of 21,965 snails was collected from CPOM and rock habitat types from the two streams over the length of the study; 15,803 on CPOM and 6162 on rocks. No snails were

recorded from the substrate samples taken from sites T1 to T4 in sampling seasons 1 and 2, and only 17 snails were present in the substrate samples at the upstream control (UC1) during the first two sampling events.

The total number of snails differed between years, sites and streams (Figure 4.2). Overall snail abundances were greater at the control sites (UC1, C1 and C3) and at the upstream treatment site (T4), while the uppermost sites on both streams recorded the most snails in their respective streams. The mean snail abundance varied between sites on each stream; likewise, the abundance per stream differed (Figure 4.3). The mean number of snails for the combined treatment sites (T1 to T4) was higher (533, SD = 406.8) than for the control stream sites (449, SD = 298.8), a *t*-test found this not to be significant at $p = 0.758$. Site UC1 on the treatment stream had the highest mean abundance of 1722 (SD = 598.9, SE = 267.9) snails, a figure between 2.8 and 18 times greater than any other site. The 95 % standard error confidence intervals represented on figure 4.3 suggest a real difference in the mean sub-population sizes at sites UC1, T4 and T3, whereas natural variation may be responsible for the mean sub-population sizes at T1 and T2. The low numbers of snails recovered from T3 and small standard error indicates there is also a real difference in the mean abundance of snails at this site, as is the case at C2 in the control stream.

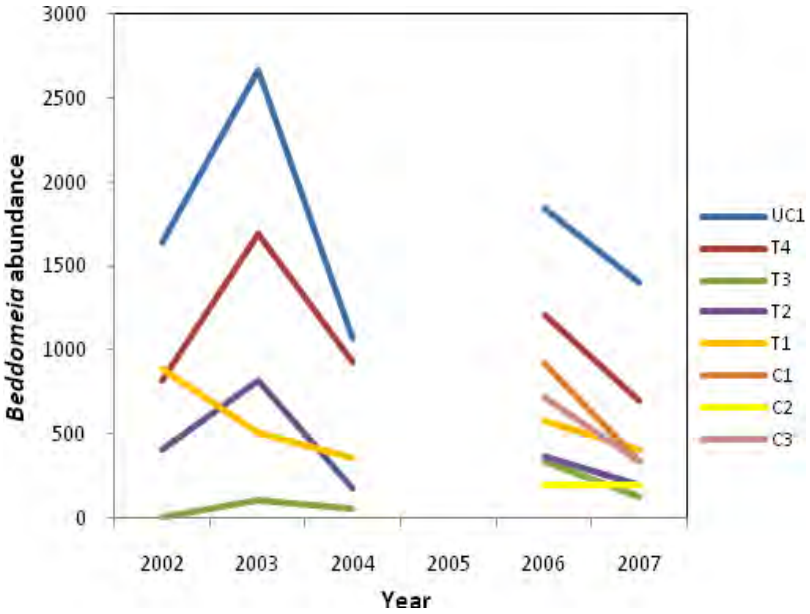


Figure 4.2. Trends in total abundance of *Beddomeia* spp. per site across the study period.

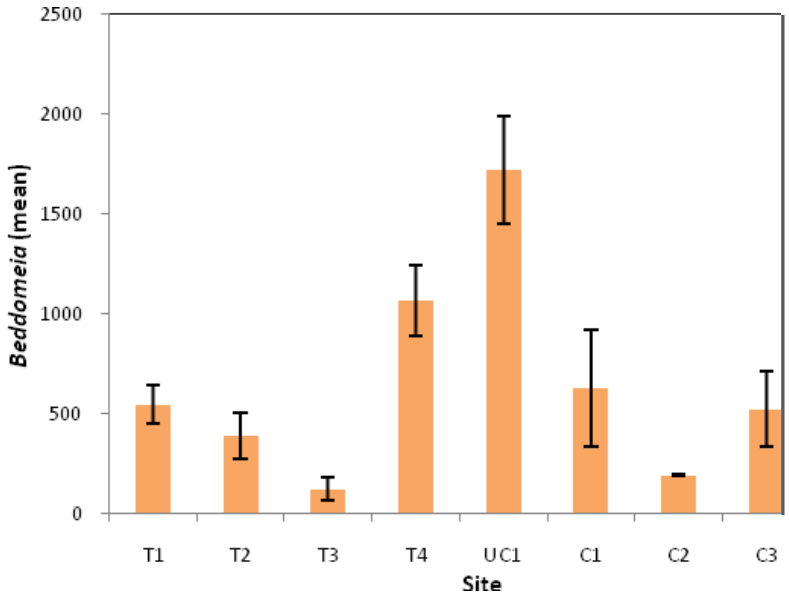


Figure 4.3. Mean abundance of *Beddomeia* spp. (± standard error) per site during the study period (mean of combined habitat data per site over five sampling events for treatment stream (T1 – T4 and UC1) and two sampling events for control stream (C1 – C3)).

Comparable patterns of year-to-year population fluctuations were detected at sites on both streams during the sampling period (Figure 4.2); similarly, the relationships between snail abundance and site were consistent between sampling seasons on most occasions. A decline in snail abundance was recorded in 2004 and it declined again in 2007. These patterns were detectable using total abundance as well as the standardised snail density data for CPOM (no. per tray) and rock habitats (no. cm^{-2}), with the exception of the CPOM data for UC1 (Figures 4.4 and 4.5).

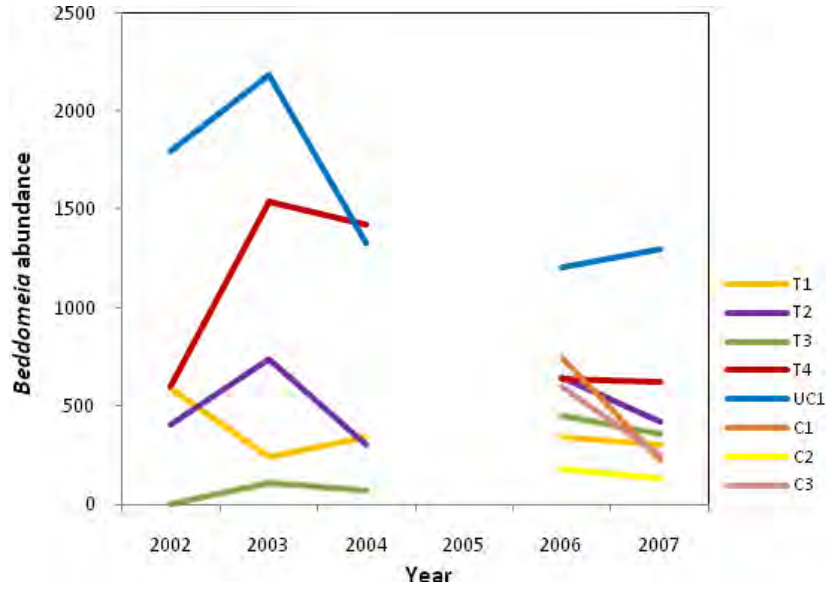


Figure 4.4. Standardised densities of *Beddomeia* on CPOM (no. per tray) by site by site per sampling event.

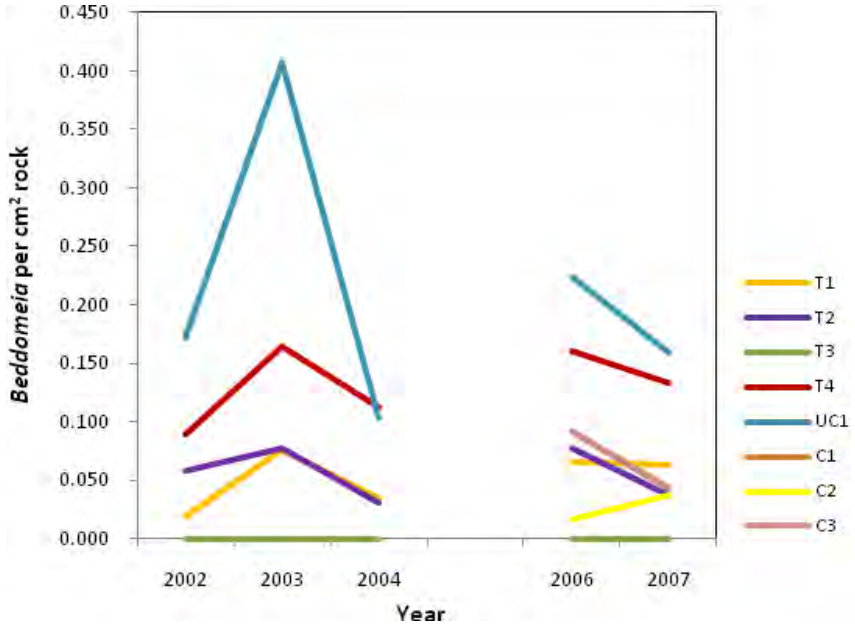


Figure 4.5. Standardised densities of *Beddomeia* on rocks (no. cm^{-2}) by site per sampling event. C3 overlays C1 as densities are the same.

4.3.1.1 Snail abundance and habitat type

Allochthonous material (CPOM) harboured 72% of the total number of *Beddomeia* recorded (Table 4.3); however, direct comparison of the habitat data to identify habitat preference was not possible due to differences in surface areas of habitat sampled.

Despite the severely burnt condition of in-stream allochthonous material at the treatment sites, *Beddomeia* spp. were visible on some branch material from the first sampling season at two treatment sites and egg capsules were recorded on burnt logs at T4 from 2004 onward. Patterns of snail densities per cm^2 rock surface area were similar in both the control and treatment streams, declining in a downstream direction and remaining low at the downstream site T1, in mature forest. Snail density per tray of CPOM was greatest at UC1, and variable at other treatment stream and control stream sites, but showed evidence of increasing at T3 from 2006. Volumes of CPOM recovered are presented in Appendix A.

Table 4.3. Total number of *Beddomeia* spp. recorded on each habitat type by sampling event.

	2002		2003		2004		2006		2007	
Site	CPOM	Rocks	CPOM	Rocks	CPOM	Rocks	CPOM	Rocks	CPOM	Rocks
T1	738	151	238	268	259	100	343	231	225	185
T2	268	135	739	74	153	25	216	152	139	61
T3	0	0	106	0	53	0	340	0	120	0
T4	398	422	1535	158	711	215	640	566	413	287
UC1	1199	441	2186	476	994	74	1203	635	976	424
C1							594	325	179	156
C2							135	58	65	131
C3							450	264	188	148

4.3.2 Relationship between snail abundance and habitat variables

The full model used in the regression analyses was:

Response variable ~ stream + stream no. + year + slope + logCatchment + ans.age + riparian.slope.1 + riparian.slope.2 + CPOM.instream + boulders + cobbles + pebbles + depth + width + pc.riffle + pc.pool + flow + stream.order.

Descriptions of each variable is presented in Table 4.2. Additional explanation of terms in model: pc.pool = percentage of sub-site containing pools; pc.riffle = percentage of sub-site containing riffles; and yr = sampling event.

The number of contributing explanatory variables in the models recovered using the three *Beddomeia* response variables differed slightly; riparian slope, CPOM instream and stream order were not consistently retained in the different models. Differences were more pronounced when „stream order' was included in the full model, as stream order and additional variables (CPOM instream and riparian slope) were then identified as significant in some models, whereas by omitting stream order, fewer variables, only site and forest stand age, were included in each of the models and the R^2 values were poorer. Differences in the models produced incorporating or excluding stream order suggests stream order is highly correlated with some other variables in the model, so that when it is included it suppresses their influence, allowing CPOM and riparian slope to be retained. However, models including stream order are used, as there is sufficient evidence to suggest this is a strong predictor of snail abundance (Chapter 3 data, Table 3.3) (see Section 4.4.3).

The final CPOM analyses identified three parameters, site, stream order and log of forest stand age, as possible explanatory variables for variation in snail abundance, where „stream order' was included in the model (Table 4.4), but when excluding „stream order', only two variables, site and log of forest stand age contributing to the final model, although the R^2 value was then much reduced.

For CPOM analyses the model with the highest R^2 value ($R^2 = 0.701$, adjusted $R^2 = 0.668$), identified site number, stream order and forest stand age as explanatory variables, whereas removing „stream order' from the analysis limited the model to two explanatory variables and reduced the R^2 to 0.489. Analyses of the data using transformed total *Beddomeia* on CPOM and standardised *Beddomeia* per tray of CPOM data failed to identify any variables responsible for the data. In each instance the relationship between snail abundance and stream order, site number and the log of forest stand age was positive, indicating snail preference for uppermost sites on smaller streams and higher forest cover.

Table 4.4. ANOVA for the stepwise regression model prediction of three contributing explanatory environmental variables using snails per tray of CPOM as the response variable (R^2 = 0.701, adjusted R^2 = 0.668). Observations (Sum Wgts = 31).

Source	DF	SS	MS	*F* Ratio	*P* > *F*
Model	3	6352066.5	2117356	21.097	<.0001
Error	27	2709775.0	100362		
C. Total	30	9061841.5			

Parameter Estimates

Term	Estimate	SE	*t* Ratio	*P*>|*t*|
Intercept	-2071.637	457.342	-4.53	0.0001
Site No	500.273	69.344	7.21	<.0001
Stream order	792.013	195.432	4.05	0.0004
Forest stand age*	300.413	112.715	2.67	0.0128

* Age data were transformed using ASIN(SQRT(X/100)).

Step-wise regression conducted on the rock habitat data using standardised snail density (no. cm^{-2}) as the response variable identified variables responsible for the data at $p < 0.05$: stream order, forest stand age, riparian slope and CPOM instream were useful explanatory variables (Table 4.5). All factors were positively correlated with snail density. The models produced excluding stream order had poorer R^2 values.

Fewer variables were identified as contributing to the model explaining the snail data in the analysis using total snail abundance as the response variable. The same five parameters identified in the previous analysis were also recognised as explanatory factors, but the fit of the model was better (R^2 = 0.791, adjusted R^2 = 0.749). Omitting „stream order' from the full model, the R^2 values were much lower (R^2 = 0.539, adjusted R^2 = 0.506), indicating a poorer model, but one still accounting for 50% of the variability in the data. As the variables recovered, and the relationships between them and snail abundance were the same, the results are not presented here for brevity.

Table 4.5. ANOVA for the prediction of stepwise regression model identifying five contributing explanatory environmental variables using standardised snails on rocks per sample (R^2 = 0.767, adjusted R^2 = 0.7199). Observations (Sum Wgts = 31).

Source	Df	SS	MS	*F*	*P(>F)*
Model	5	0.165	0.033	16.422	< 0.0001
Error	25	0.050	0.002		
C. Total	30	0.215			

Parameter Estimates

Term	Estimate	SE	*T*	*P(>|t|)*
Intercept	-0.503	0.085	-5.93	<.0001
Site	0.076	0.0099	7.74	<.0001
Forest stand age	0.001	0.0002	4.15	0.0003
Riparian slope 1	0.005	0.002	3.25	0.0033
CPOM instream	0.010	0.004	2.57	0.0165
Stream order	0.159	0.031	5.21	<.0001

The analyses above were conducted on the full dataset which included both the control stream and treatment stream data. However, running similar analyses on the treatment stream data alone, failed to identify „age of forest stand' as a significant explanatory variable. CPOM and total *Beddomeia* response variables identified only two factors contributing to the model; site and catchment size, whereas the variables site, catchment size and CPOM instream were the significant factors explaining snail density per surface area of rock.

4.3.1.2 Testing for variability between treatment sites

A two-way unreplicated ANOVA of the CPOM data (a surrogate for overall population abundance) was used to test for linear, quadratic and higher order trends across sites or years, by treating the years within the sites as the replicated observations, and the sites as an ordered, fixed factor with 5 levels (Table 4.6). A plot of the square-root transformation of *Beddomeia* data on CPOM (no.tray), all sites, with T3 omitted, reveals a linear downstream decline in snail densities on CPOM (Figure 4.6) that was not detectable when T3 is included (Figure 4.7). This analysis assumes no interaction, i.e. the behaviour of the trend across sites is consistent over time and is only possible if T3 is omitted from further analysis, but that the trend is not consistent, i.e. some interaction is occurring.

Site T3 displayed different physical attributes to the other treatment sites, being of reduced slope, possessing a poorly defined channel and being infilled with large volumes of deposited gravel sediment. The sediment deposition at T3 was greatest in 2002, slowly reducing over subsequent years. By omitting T3 from the analysis a linear pattern is revealed. However, the interaction cannot be estimated due to lack of replication within sites within years.

Table 4.6. ANOVA table for the test of polynomial terms.

	Df	**SS**	**MS**	***F***	***P(>F)***
Site	3	1150.20	383.40	11.602	0.0007
Year	4	136.46	34.11	1.032	0.4307
Residuals	12	396.54	33.05		

	Estimate	**SE**	***t***	***P(>|t|)***
(Intercept)	27.933	1.285	21.731	5.28e-11***
Site				
Linear	14.723	2.571	5.727	9.50e-05***
Quadratic	3.444	2.571	1.339	0.205
Cubic	-1.190	2.571	-0.463	0.652
Year				
Linear	-4.878	2.874	-1.697	0.115
Quadratic	-1.399	2.874	-0.487	0.635
Cubic	2.420	2.874	0.842	0.416
year^4	-1.581	2.874	-0.550	0.592

There was no significant variation in snail abundance between years ($F_{(4,12)} = 1.03$, $p = 0.430$), but there was a linear trend across the sites from upstream to downstream ($F_{(1,12)} = 32.80$, $p < 0.0001$) (Table 4.6). There was no evidence for any quadratic or cubic trends (both $p > 0.2$). The increasing abundance at site T3 over time may be explicable in terms of changes observed in the amount of fine sediment and available habitat, the slope of the site being less than at other treatment stream sites, so that it acted as a bench, accumulating high levels of sediment, burying all rock and most CPOM during the first three sample seasons, subsequently reducing habitat availability.

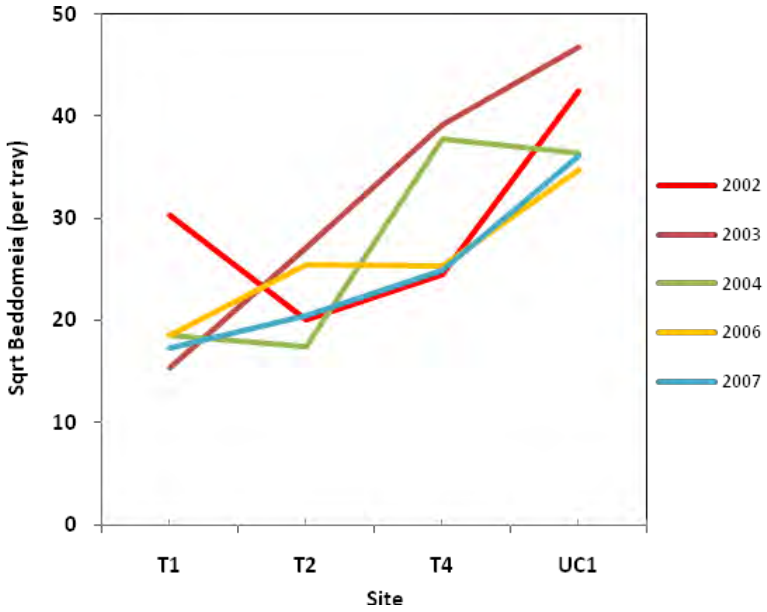


Figure 4.6. Plot of square-root transformation of *Beddomeia* density (no. per tray CPOM) by site (excluding site T3).

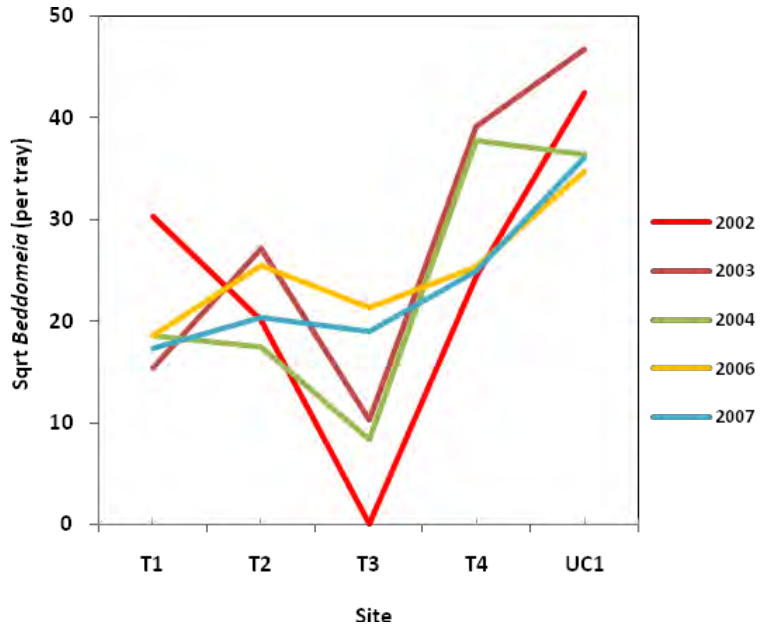


Figure 4.7. Plot of square-root transformation of *Beddomeia* density (no. per tray CPOM) by site, all sites included.

4.3.1.3 Testing for variability between controls

A two-way ANOVA of the control site data suggests that the sites differed between themselves, but no evidence of any difference between the years (Appendix B). As expected, the diagnostics were not very insightful due to the small sample size, however, trends in the data showed that site C2 had fewest snails of all the sites, while C3 was intermediate between C1 and C2, and UC1 was more than three times greater than the highest abundances in the control stream. A plot of the square-root transformation of *Beddomeia* on CPOM (snails per tray) data by site illustrates the variability between control sites and years, while UCI retained highest overall densities compared to the other control stream sites (Figure 4.8).

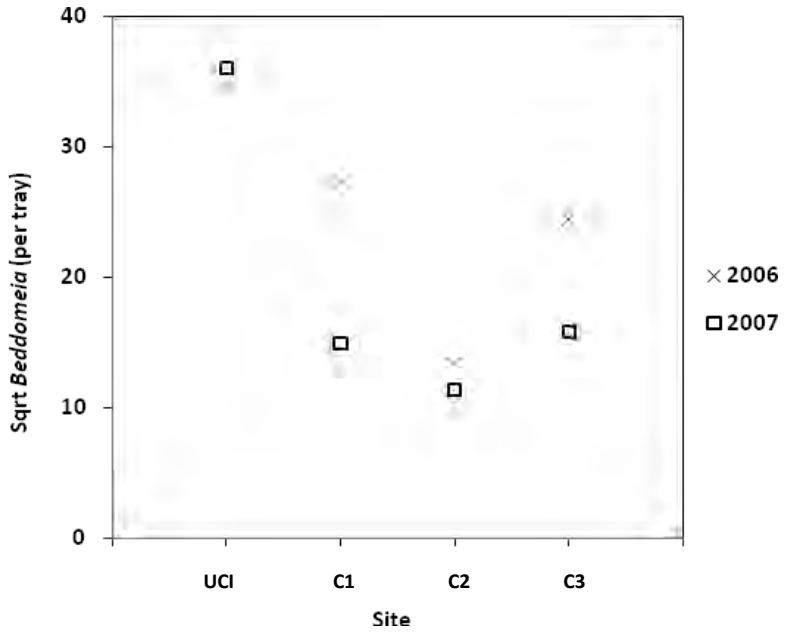


Figure 4.8. Plot of square-root transformation of *Beddomeia* density (no. per tray CPOM) by Control site.

4.3.3 Population characteristics

Morphotypes

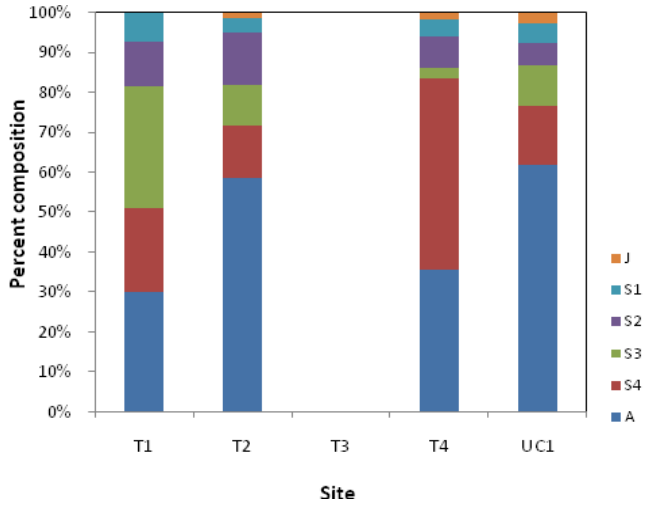
Four sympatrically occurring morphotypes of *Beddomeia* were recorded in both streams during this study. An average of three morphotypes was present per site, while two was the minimum number recorded and all four morphotypes co-occurred at four sites. Although the relative abundance of each morphotype in the sub-population at each site differed between

years, the pattern of dominance remained static throughout the study. Morphotype 6 was the most abundant at all sites, on all sampling occasions, contributing between 49% and 85% to the total population. The next most abundant was morphotype 1, which also occurred at each site, on all sampling occasions. The two morphotypes, 3 and 2, representing the extremes of shell variation amongst the four morphotypes, occurred less frequently; morph 3 present at six sites and morph 2 at only four sites: both morphotypes occurred in low densities. There was no site-to-site pattern in the proportions of morphotypes.

Age structure

The proportions of *Beddomeia* spp. by age category differed between the upstream control (UC1) and treatment sites on the treated stream during the first two years of the study. Population structure differed between all sites throughout the study period, but most particularly at sites T2 and T3 where only a proportion of age cohorts were present in 2002 to 2004, for example at T3, no snails were recorded in 2002, adults and S4 generations were present in 2003 while no juveniles or S2 cohorts were recorded in 2004 (Figure 4.9). However, at other sites, on both streams, the mature cohorts (adult and S4 generation *Beddomeia*) contributed between 50 to 80% to the population, but percentage composition was constant at sites on the control stream. Site UC1 maintained high proportions of mature cohorts over the sampling period (Figure 4.10), while an unusually high percentage of juveniles was recorded at all treatment stream sites in 2006 (Figure 4.9). All age cohorts were recorded at each control site during 2006 and 2007; sites C1 and C2 recording similar proportions in both years, as did C3 and UC1 (Figure 4.11).

Structure of the population during the two periods of population decline did not alter significantly, with most age cohorts present at four sites in 2004 and all but T1 containing juveniles in 2007. The major difference between the population structures during the two population declines was the detection of juveniles which were recorded at only one site in 2004 compared with four sites in 2007.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Percent composition
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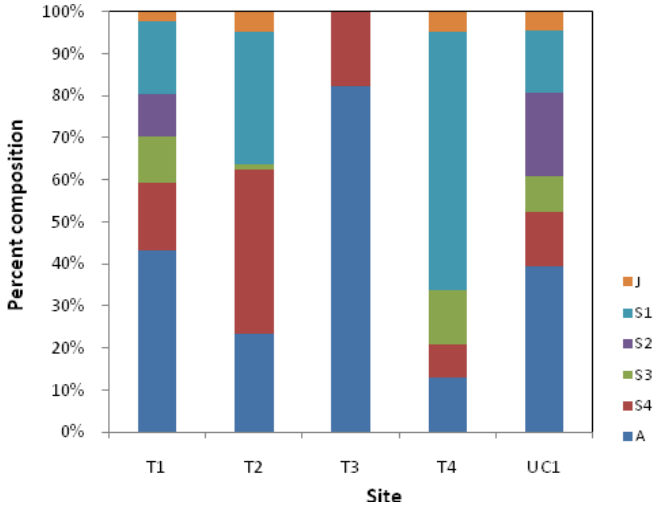
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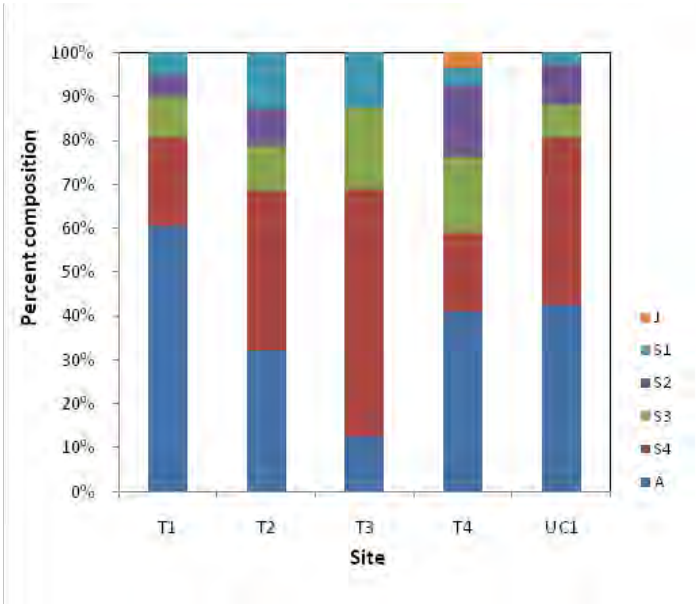
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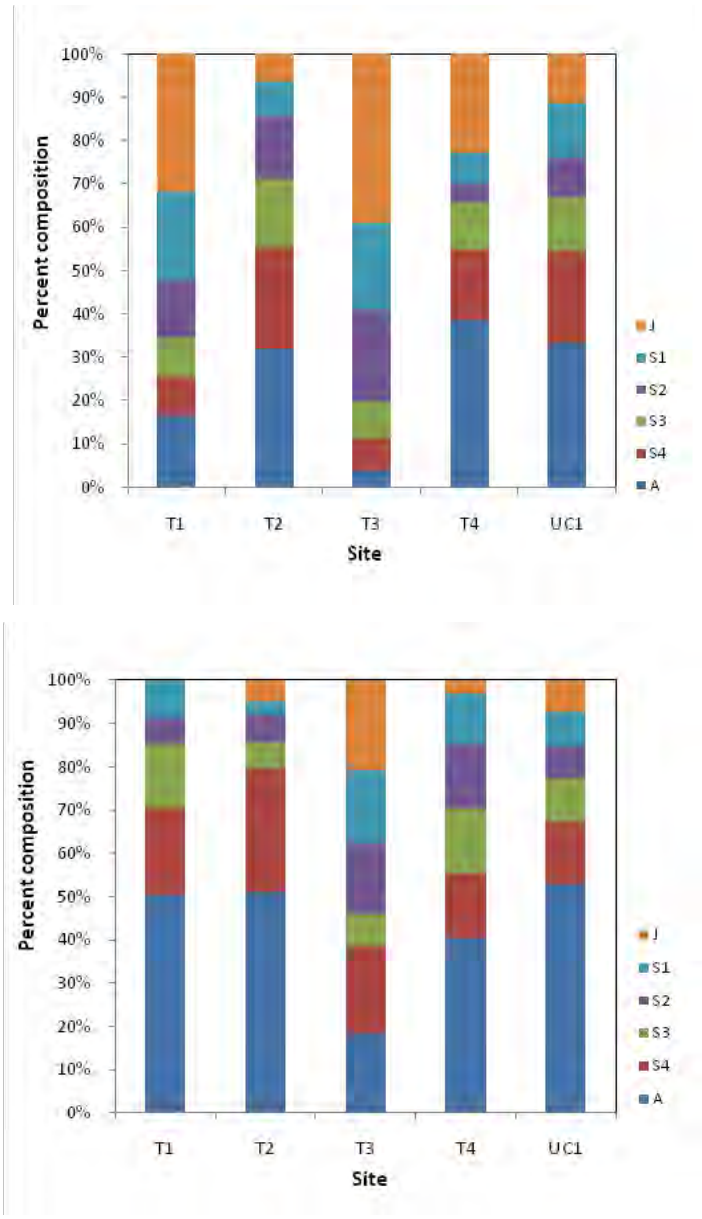
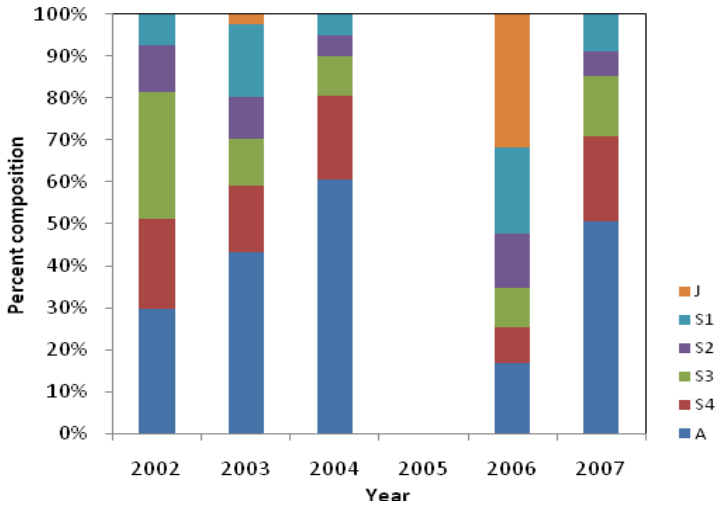


Figure 4.9. *Beddomeia* spp. population structure in the treatment stream sites throughout the study period from 2002 (uppermost) to 2007 (above). T1 was located in unharvested forest downstream of the harvest operation area, T2 – T4 located within the harvest area, and UC1 was in unharvested forest upstream of the harvest area. Population structure was separated into six size classes: J = juvenile, S1 – S3 progressively larger classes, S4 = subadult, and A = adult.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Percent composition
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Year
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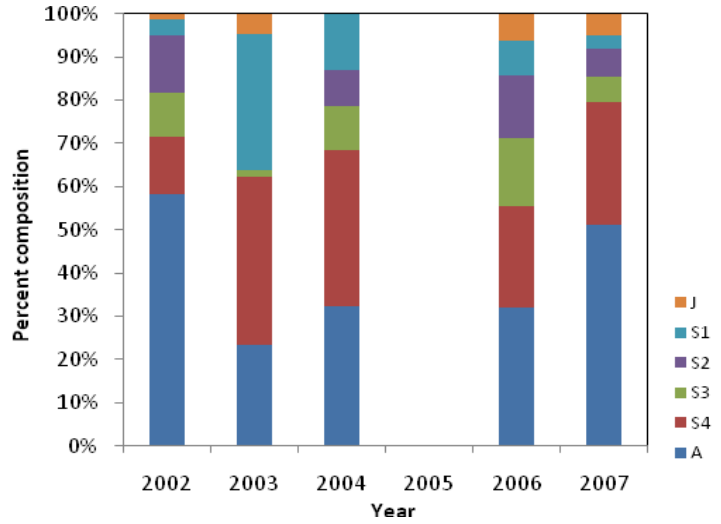
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J
S1
S2
S3
S4
A

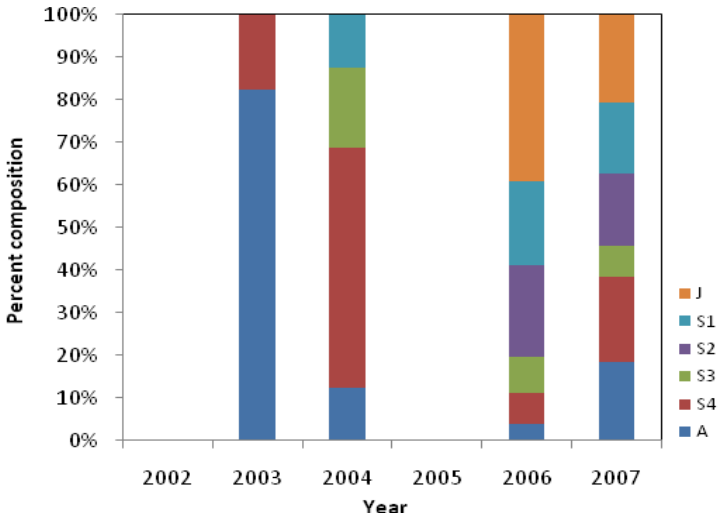

100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Percent composition
2002
2003
2004
2005
2006
2007
Year
J
S1
S2
S3
S4
A

Figure 4.10. *Beddomeia* spp. population structure per treatment stream site over five sampling events. Charts are positioned from T1 – T4 with UC1 lowest. T1 (uppermost) was located in unharvested forest downstream of the harvest operation area, T2 – T4 located within the harvest area, with T2 most downstream and T4 uppermost, and UC1 (below) was in unharvested forest upstream of the harvest area. Population structure was separated into six size classes: J = juvenile, S1 – S3 progressively larger classes, S4 = subadult, and A = adult.

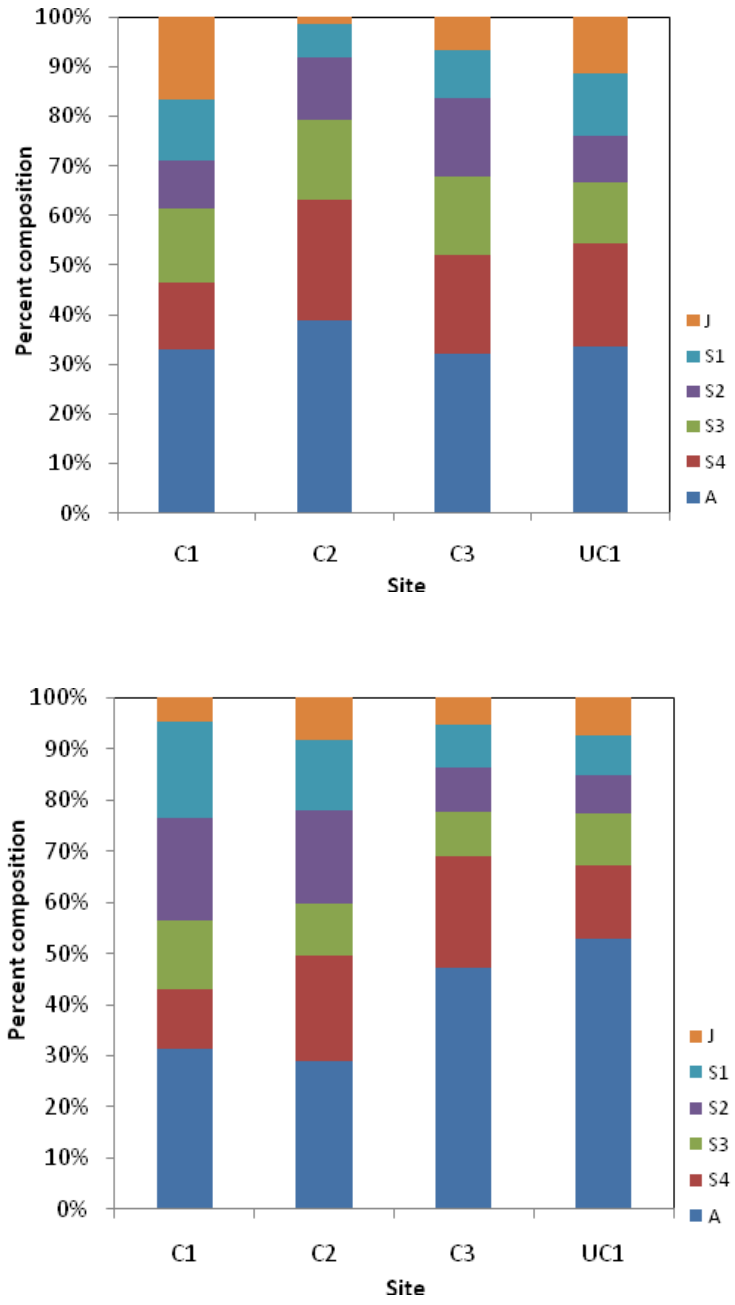


Figure 4.11. *Beddomeia* spp. population structure in the control sites throughout the study period. UC1 was the pseudo-control located in unharvested forest downstream of the harvest operation area, C1 - C3 were control sites on the control stream, with C1 most downstream and C3 uppermost. UC1 was in unharvested forest upstream of the harvest area. Population structure was separated into six size classes: J = juvenile, S1 – S3 progressively larger classes, S4 = subadult, and A = adult.

4.4 Discussion

In this retrospective study, the effects of one high level disturbance, cable-harvesting, on snail population abundance and structure were examined. The results illustrate natural variation in abundances of *Beddomeia* spp. within and between sites on the same stream and between control sites on the two streams, and suggest limited impact on the *Beddomeia* abundance resulting from the harvest operation. However, there were some observed changes in population age structure and abundances between disturbed and natural sites suggesting cable harvesting does have some impact on snail populations, at least during the short to mid-term (0 – 10 year period).

4.4.1 Abundance of Beddomeia in headwater streams

Natural variability in stream ecosystem functioning and microhabitat distribution is widely recognised (Gomi *et al.* 2002), influenced by patch dynamics (Pringle *et al.* 1988) and is dependent on a suite of environmental factors including slope, flow, substrate, allochthonous input, sediment movement, stream depth and disturbances. Such variation makes determining the causes of changes to stream invertebrate abundances difficult. In this study, the treatment stream showed an unusually high, but variable, density of snails compared to similar streams in both the Groom River and Castra Rivulet catchments (Chapter 3), and the snail population density of the treatment stream was higher than in the control stream. The extent to which this high abundance of snails may have affected the results of this study is unknown, but some influence might be expected.

Despite the high densities of snails in the treatment stream, some trends in the data were detectable. A linear trend of decreasing snail abundance in a downstream direction was evident in the treatment stream when the ‚atypical' site T3 was removed; however, due to the limited number of sites on the control stream, no similar pattern could be identified. The uppermost site on the treatment stream (UC1) contained the highest abundance of snails whereas, on the control stream, the location of the highest abundance was inconsistent between years, alternating between site C3 and C1. The lowest abundances varied between the three downstream sites on the treatment stream.

4.4.2 Comparison of snail abundance in harvested and control streams

Overall, the snail abundance in the treatment stream was higher and most treatment sites recorded higher abundances than the control stream sites, the one exception being T3, which continued to record the lowest snail numbers for the first three years of the study and remained low in 2006 and 2007. Such differences in observed snail abundances between adjacent, or nearby headwater streams, and within streams, is not unusual; for example, multiple examples of in-stream variation were reported in the two catchments used in this thesis, (Chapter 3). Natural variation in snail abundances between sites was also recorded in the control stream, site C2 containing fewer snails than other sites, although overall abundance remained high in all three sites.

Observed differences between the control sites, particularly the upstream sites C3 and UC1, reflected natural variation, adding support to the use of UC1 as a relevant control for the treatment stream data, but also implying that caution is necessary when interpreting the data using only one control and only limited data points. To illustrate this, natural variation in population abundance could be seen between sub-sites within UC1, in one instance more than doubling the number of snails from 1068 to 2662 within 5 m of stream, probably as a result of changes in rock and CPOM availability. A further example was recorded in the control stream, where the abundance of snails on CPOM reduced by more than half at each site between 2006 and 2007. Such variation can be minimised by adequate replication in sampling and examining averages of the data to detect trends.

While there are observable spatial distribution patterns in the *Beddomeia* data for the two streams, no such temporal linear trends in snail abundance are apparent across the sampling period. Although there is evidence to suggest that some sites within the harvested area did show an increase in snail abundance between 2002 and 2007 (T2 and T3), this pattern was not repeated elsewhere. There is, however, some evidence to support an overall population decline in the treatment stream in 2004 and again in 2007, in both instances a reduction in snail abundance was observed across all sites and changes to population structure were recorded (see Section 4.4.4). This pattern of decline was also observed in the control stream in 2007, but unlike in the treatment stream, there were minimal changes in the population structure. While this may appear to be due to sampling error, this is unlikely due to strict adherence to the sampling methodology.

4.4.3 Relationship between snail abundance and habitat variables

Naturally occurring variation in snail abundance between study streams can introduce errors into the analyses by recognising and including differences which then influence the analysis. This is highlighted by the two approaches to data analysis, which result in considerably different interpretations of the effects of harvesting on the snail population.

Analysis of the combined stream dataset identified five factors; site, stream order, age of forest, riparian slope and percentage of CPOM instream, as significantly influencing snail abundance in this study. Site and forest stand age were consistently predicted as contributing explanatory environmental variables common to each analysis, whereas the additional variables, riparian slope, CPOM instream and stream order were recognised as explanatory factors using the CPOM and „total *Beddomeia*' datasets. The identification of site as an important explanatory predictor of snail abundance is supported by the findings of the two-way ANOVA for the treatment stream, which recognised sites as significantly different. However, no clear distinction between the control and catchment streams was identified by the regression analyses of the combined streams dataset, with stream failing to be recognised as a contributing explanatory factor.

Additional regression analysis conducted on the treatment stream subset of data resulted in a rather different outcome, recovering only two significant variables, site and catchment size, contributing to the model. This result was confirmed by two-way ANOVA which found site to be the only significant variable explaining the data, while a plot of the square-root transformation of *Beddomeia* on CPOM (no. per tray) by site revealed a negative linear relationship between snail density and site, when the atypical site T3 was removed, implying a decline in snail abundance with increasing catchment size.

Although mean snail abundance in the control stream was lower than the treatment stream, the mean snail abundance of control stream sites fit within the range reported for the treatment stream. In addition, two of the control sites (C1 and C3) contained higher snail densities than two treatment stream sites (T2 and T3). Thus, inclusion of the control stream data appears to have had two effects: (1) the high C1 and C3 abundances in mature forest has driven the recognition of forest age as an important predictor of snail abundance, and (2) it had a masking effect on identifying differences between streams.

Variation between sites was detected in both the treatment stream and the control sites. Using the CPOM snail density data as a surrogate for the entire population dataset, the treatment stream sites were found to be significantly different from each other, and a linear trend in *Beddomeia* spp. density along the treatment stream was recognised in the analyses. If an impact of the harvest operation were present, some level of downstream recovery should be expected in the treatment stream data. However, snail numbers remained high at the T4 treatment site throughout the study, and with the exception of an increase in snail abundance at T1 in 2002, no sign of recovery downstream of the harvested area was observed, despite the lower abundances of snails observed within the treatment area (at T3 and T2) throughout the early years of the study. This suggests either that the data reflect a natural population decline only minimally affected by the harvest operation, or that to detect recovery from a decline in snail abundance resulting from the harvest requires sampling further downstream.

The study was unable to critically examine the significance of apparent preferences for habitat type; however, results suggest that snail presence and abundance was partially influenced by habitat availability. CPOM and rocks were identified as the principal components of habitat for *Beddomeia* spp. after the first two years of sampling, with CPOM and rocks containing more than 99% of the snails, thus substrate sampling was subsequently removed from the study. On average, CPOM samples harboured 2 ½ times more snails than rocks, but differences in surface areas of the habitat types sampled is likely to influence this finding. *Beddomeia* were recorded on the underside of rocks throughout the study sites; however, the availability of suitable rock habitat was shown to be a limiting factor for snail abundance at one site, for both habitat types. The absence of rocks at T3 appears to have constrained the recovery of *Beddomeia* spp. on CPOM at that site, by limiting the ability of the site to capture and retain re-distributed CPOM and the failure to retain the majority of finer „leaf and branch' elements of CPOM at the site which frequently lodge between rocks.

The treatment stream in this study was unusual in that it contained an extraordinarily high abundance of *Beddomeia*, more like hydrobiid population densities found in spring-snails associated with artesian springs in the U.S.A., e.g. *Assiminea infima* (Sada 2001) or lentic populations of *Hydrobia ventrosa* in Tunisia (Casagranda and Boudouresque 2002) than other Tasmanian forest headwater streams. Such high densities have not previously been recorded from headwater streams in Tasmania (Davies and Cook 2002; Chapter 3; FPA unpublished data) and were not known at the start of this study. The results may lead to the conclusion that *Beddomeia* spp. populations survive relatively intact (although with reduced abundance) after cable-harvesting events. But this outcome may simply reflect the unusually

high snail density, and may not be observed in lower abundance populations that have limited capacity to recover from population decline, or indeed in populations of other *Beddomeia* species. Harvesting operations which are unable to leave stream headwaters relatively intact may also produce different outcomes. Therefore, caution is needed in interpreting the data, and their application to other situations, as it may lead to inappropriate management decisions and conservation outcomes.

4.4.4 The effects of cable-harvesting on **Beddomeia spp.** *population structure*

Evidence from the population composition data suggests that an initial decline in the snail abundance is likely to have occurred in the treatment stream following the harvesting event, although by how much is unknown. The most notable changes to population structure were observed at treatment sites with low population densities, where only adult and S4 cohorts were present in the early years, in particular site T3. Successive sampling events demonstrated population structural recovery at such sites in the treatment stream by 2007, despite the stream suffering a population crash in 2004 and again in 2007. Indeed, most sites in the treatment stream had shown some evidence of population structure recovery prior to 2007, however, the delay in recognition of total stream recovery was due to limited reproduction as a result of low population numbers at some sites; such sites also displayed a lack of consistency between the relative proportions of age cohorts present and detection of reproduction (presence of juveniles) between successive sampling seasons.

Preliminary signs of population recovery were discernable in the treatment stream by the second year of the study (2003), when greater proportions of juvenile and S1 cohorts were recorded at two of the treatment sites (T2 and T4); however, these did not translate to proportionally similar numbers of older cohorts (S2 and S3) at the adjacent sub-site in subsequent years. The overall rate of population recovery was low, due in part, to a combination of low fecundity (evident in the population structure), longevity (slow maturation), habitat availability and poor dispersal capabilities of *Beddomeia* spp. (Ponder *et al.* 1993; Richards unpublished data). However, by 2007, eight years after the forest was harvested and seven years after the riparian vegetation was burnt, the population age structure at all sites excepting T3 converged, showing similar patterns of cohort compositions and proportions. While site T3 differed from other sites, it also began to show strong recovery by 2007 with the arrival from upstream of larger, decaying CPOM harbouring healthy families of snails.

Several morphotypes of *Beddomeia* were recorded living sympatrically in the study streams. The morphotypes detected and their pattern of dominance are consistent with the findings from the adjacent river catchment studied in this thesis (Chapter 3). Similar anatomical variation was reported in Chapter 3 (see Chapter 5), indicating that the current speciation of the *Beddomeia* complex is likely to be incomplete. While variability in the presence and abundance of morphotypes was evident, no trend relating to the influence of the harvesting operation on individual morphotypes could be discerned. The pattern of morphotype dominance remained static throughout the study, due to the high proportions of the dominant morphotype at all sites, whereas the sampling methodology applied did not allow any discrimination between habitat requirements of individual morphotypes. Further work is required to address the question of speciation within the *Beddomeia* genus.

4.4.5 Impact of cable-harvesting on snail populations and value of buffer strips

This study represents one of the few species-specific studies on the impacts of forestry on aquatic invertebrates in Australia, although some studies have been carried out in the Canada on vertebrate species, such as the tailed frog (*Ascaphus truei*) in British Columbia and salamanders in the Pacific Northwest, U.S.A. (Dupis and Steventon 1999, Olson *et al.* 2007). The results complement the findings of retrospective studies on general invertebrate fauna in streams following harvesting conducted in Australia (Davies *et al.* 2005a, Davies *et al.* 2009), and studies of the effects of logging on macroinvertebrate communities and habitat in Western Australia (e.g. Davies and Nelson 1994, Growns and Davis 1994), Canada (e.g. Fuchs *et al.* 2003, Moldenke and Ver Linden 2007) and the U.S.A. (e.g. Cole *et al.* 2003, Danehy *et al.* 2007).

Buffer strips are recognised as important contributors to maintaining stream water quality and ecosystem health. The removal of riparian overstorey leads to compositional changes in allochthonous material (Dahlstrom and Nilsson 2004, Watson 2004). Supporting evidence from coniferous forests studied in the U.S.A. have shown that whilst there are proportional increases in CPOM following harvesting events, structural changes in CPOM also occur, shifting from complex CPOM containing decomposing leaf material to a simpler structure composed mainly of larger woody debris (Ralph *et al.* 1994, Gomi *et al.* 2001). Similar studies in Australia have also identified a transition to larger branch material following harvesting (Davies *et al.* 2005b), while Watson (2004) reported an increase in CPOM volume as a result of forest harvesting in areas harvested six years prior.

By its very nature, steep country cable-harvesting and the associated post-harvest regeneration burning is likely to cause most disturbance to internal headwater streams, through both operational constraints (forest removal, mechanical and secondary disturbance to riparian vegetation and operational burns) and impacts on stream food resources. Burning of operational waste to create seed beds (regeneration burning) further reduces complexity of the allochthonous material, removing all leaf, twig and small branch material and leaving only severely burnt, larger branches to be deposited in streams, the majority of which fails to have water contact for several years. The rate of decomposition of this larger branch matter is relatively slow. Gomi *et al*. (2001) reported that large woody debris (LWD) remained in Alaskan streams 37 years after harvest. It may also be some time before the material begins to accumulate biofilm in-stream. However, the results of this study provide an alternative viewpoint, since snails reliant on biofilm (periphyton layer likely to be dominated by bacterial species (Spiers 2003)), were recorded on submerged, burnt wood, during the first sampling event, two years after the operational regeneration burn, and egg capsules observed on the wood the following sampling season. This suggests that burnt wood is not a hindrance to establishment of biofilm and subsequent colonisation of *Beddomeia* in the short to medium term, providing that the wood is permanently inundated and the snail population can utilise alternate resources, such as rocks, until the biofilm becomes established.

The almost complete removal of riparian vegetation from the harvest area and burning of the residual harvest debris in this study caused substantial changes to the CPOM composition at treatment sites T2-T4 that remained observable for the duration of this study. Age of forest was identified as an explanatory factor of snail abundance, suggesting that some impact on the snail population did occur. Despite this dramatic change, *Beddomeia* persisted in the treatment stream, although there is evidence to suggest that a population decline did take place around the time of the operation burn. The exact nature of the population decline is unclear, but it was sufficient to remove snails from some areas (e.g. site T3) at least for the short-term, and to reduce the snail abundance at some other sites.

This cable-harvest operation was atypical in that it occurred mid-slope, leaving the headwaters of the stream with an intact forest cover. It is likely that where higher snail densities occur there is a propensity for proportionally more snails to be transported downstream on CPOM during rainfall events, thus supplementing the downstream population and reducing the length of recovery time which might otherwise be needed in streams with lower population densities. Retention of upstream habitat might also have prevented a much greater impact on the snail population by continually contributing CPOM. The higher than

expected snail abundance at the upstream control (UC1) and in the upstream treatment site (T4) lends support to this hypothesis. Interestingly, in 2004, there was one record of higher snail abundance at T4 than at the control site upstream; however, on this occasion increased levels of well structured CPOM were recorded at this site, likely to have resulted from a heavy rainfall event during the previous few days, which transported large volumes of CPOM downstream. This is further supported by the observations of high residual turbidity recorded downstream during sampling in 2004, increased levels of leaves in the CPOM and increased stream depth (in the order of 200%).

The implication for smaller *Beddomeia* spp. populations in similar atypical harvest operations is that while snail dispersal can be enhanced by CPOM movement, headwater stream densities are likely to be reduced, and for very small populations the very real possibility of local extinctions arising if large amounts of CPOM are transported too far downstream. Further, in situations where stream headwaters are totally compromised (harvested and burnt leaving no stream buffer), these smaller populations are more likely to face extinction due to changed stream conditions and lack of allochthonous input in the years immediately following harvest.

As demonstrated in this study, headwater streams display unique characteristics and within them *Beddomeia* populations respond to habitat availability and site-specific conditions. Careful consideration of the outcome must be undertaken prior to applying these findings to other situations. The implications here for forest procedures are that such streams may require site-specific management, particularly where lower density *Beddomeia* spp. populations are present.

4.5 Conclusions

Beddomeia spp. demonstrated natural variation in abundances within and between sites on the same stream and between streams, making a retrospective examination of the effects of cable-harvesting on the *Beddomeia* population difficult to interpret. Some differences between treatment and control sites could be discerned, but were poorly resolved. Observed changes in the population structure indicate that a decline in population abundance had recently occurred in the treatment stream, possibly coinciding with the timing of the cable-harvesting operation. Recovery of population structure was detectable by 2007, eight years after the harvest operation, however, the consequences of a retrospective study such as this

are that the initial level of impact on snail abundance remains uncertain. Three factors, site, stream order and forest stand age were identified as significant explanatory variables of the combined dataset analysis and additional factors associated with the snail data, including percentage of CPOM in-stream and riparian slope, also contribute to the explanation of variation in abundance. However, the effect of the harvesting operation on snail abundance was less apparent when the treatment stream data was analysed separately, identifying „site' and catchment size as the only explanatory factors of the data.

It is apparent that high density populations of *Beddomeia* spp. can recover from cable-harvesting events, but it is less clear how lower density populations will respond. To test these possibilities future studies should include other *Beddomeia* species and be conducted across a range of different geological types, supporting different densities of snails. Assessment of the response of low density populations to high impact landscape changes will provide further information on how *Beddomeia* spp. populations adapt to change. Results of such studies will better inform future management of cable-harvest operations.

4.6 Appendix.

Appendix A. Composition of habitat material sampled per site.

Site	Year	Rocks available (No.)			Substrate composition (%)							Cover of CPOM instream	Tray volume (fraction)	CPOM species	main type of CPOM
		Boulders	Cobbles	Pebbles	Bedrock	Boulders	Cobbles	Pebbles	Gravel	Sand	Mud (or other)				
T1	2002	2	25	18	0	2	2	2	80	10	4	2	4/5	*D. antarctica, A. moschatum*	leaves, twigs, branches
T1	2003	2	25	25	0	2	5	2	81	10	0	2	1	*D. antarctica, A. moschatum, E. regnans*	leaves, twigs, branches
T1	2004	2	20	20	0	20	20	10	45	5	0	1	1	*D. antarctica, A. moschatum, E. regnans, E. obliqua*	leaves, twigs, branches
T1	2006	2	25	25	0	2	5	5	68	20	0	2	1	*D. antarctica, Eucalyptus sp*	leaves, twigs, branches
T1	2007	2	23	20	0	10	5	2	63	20	0	2	1	*D. antarctica, Eucalyptus sp*	leaves, twigs, branches
T2	2002	5	9	4	10	5	1	1	73	0	10	1	2/3	*Eucalyptus sp*	branches (burnt)
T2	2003	0	9	3	0	90	3	2	5	0	0	2	1	*Eucalyptus sp*	branches (burnt)
T2	2004	0	8	0	0	30	5	0	60	5	0	2	1/2	*Eucalyptus sp*	branches (burnt)
T2	2006	2	11	16	0	95	2	0	3	0	0	1	1/3	*Eucalyptus sp*	branches (burnt)
T2	2007	1	10	15	0	95	2	1	2	0	0	1	1/3	*Eucalyptus sp*	branches (burnt)
T3	2002	0	1	1	0	0	0	1	90	9	0	1	1/6	*Eucalyptus sp*	branches (burnt)
T3	2003	0	1	4	0	0	1	1	73	20	5	5	1	*Eucalyptus sp*	branches (burnt)
T3	2004	0	0	0	0	0	0	0	70	25	5	10	3/4	*Pomaderris apetala, M. gunnii, Juncus sp, D. antarctica, Eucalyptus sp leaves*	leaves
T3	2006	0	1	1	0	0	0	0	80	15	5	10	3/4	*D. antarctica, A. moschatum, E. regnans, E. obliqua*	leaves, twigs, branches (burnt)
T3	2007	0	1	3	0	0	0	0	90	10	0	5	3/4	*D. antarctica, A. moschatum, E. regnans, E. obliqua*	leaves, twigs, branches (burnt)
T4	2002	2	20	9	0	5	5	5	75	10	0	2	2/3	*Eucalyptus sp*	branches (burnt)
T4	2003	0	8	8	0	1	1	1	82	10	5	2	1	*Eucalyptus sp*	branches (burnt)
T4	2004	2	13	4	0	20	10	10	50	10	0	2	1/2	*D. antarctica, A. moschatum, E. regnans , E. obliqua*	leaves, twigs, branches (burnt)
T4	2006	2	25	25	0	10	30	20	30	10	0	2	1	*Eucalyptus sp*	leaves (new), branches (burnt)
T4	2007	2	21	19	0	30	10	5	50	5	0	2	1	*D. antarctica, A. moschatum, E. regnans, E. obliqua*	leaves, branches (burnt)
UC1	2002	2	12	0	0	0	1	5	70	20	4	5	2/3	*D. antarctica, Eucalyptus sp*	leaves, twigs, branches
UC1	2003	1	8	5	0	1	1	1	90	7	0	10	1	*D. antarctica, Eucalyptus sp*	leaves, twigs, branches
UC1	2004	0	6	6	0	2	2	5	75	10	6	3	3/4	*D. antarctica, Eucalyptus sp*	leaves, twigs, branches
UC1	2006	2	18	25	0	2	5	5	60	28	0	5	1	*D. antarctica, Eucalyptus sp*	branches (burnt), leaves, twigs
UC1	2007	2	10	18	0	2	10	2	60	6	20	5	3/4	*D. antarctica, A. moschatum, E. regnans, E. obliqua*	leaves, twigs, branches
C1	2006	2	25	25	0	5	30	5	35	25	0	5	4/5	*D. antarctica, A. moschatum, E. regnans, E. obliqua*	leaves, twigs, branches
C1	2007	2	24	25	0	10	5	5	60	15	5	5	1	*D. antarctica, A. moschatum, E. regnans, E. obliqua*	leaves, twigs, branches
C2	2006	2	25	25	0	20	5	2	53	20	0	2	3/4	*D. antarctica, A. moschatum, E. regnans, E. obliqua*	leaves, twigs, branches
C2	2007	2	25	25	0	35	5	0	50	10	0	3	1	*D. antarctica, A. moschatum, E. regnans, E. obliqua*	leaves, twigs, branches
C3	2006	2	20	17	0	10	2	1	50	37	0	5	3/4	*D. antarctica, A. moschatum, E. regnans, E. obliqua*	leaves, twigs, branches
C3	2007	2	24	20	0	5	10	2	73	10	0	3	3/4	*D. antarctica, A. moschatum, E. regnans, E. obliqua*	leaves, twigs, branches

Appendix B. ANOVA table for the test of difference between control sites.

	Df	**SS**	**MS**	***F***	***P(>F)***
Site	3	549.85	183.282	9.4952	0.04848*
Year	1	58.32	58.317	3.0212	0.18057
Residuals	3	57.91	19.303		

Coefficients:

	Estimate	**SE**	***t***	***P(>|t|)***
Site				
UC1	38.079	3.473	10.963	0.00162 **
C1	-14.275	4.393	-3.249	0.04752 *
C2	-22.970	4.393	-5.228	0.01361 *
C3	-15.226	4.393	-3.466	0.04048 *
Year 2007	-5.400	3.107	-1.738	0.115

Section B Molecular phylogenetics and morphological taxonomy

This section of the thesis explores the question of taxonomic resolution within the *Beddomeia* genus. Chapter 5 provides morphological descriptions of several morphotypes discovered in studies undertaken for this thesis (Chapter 3). Chapter 6 investigates the phylogenetic relationships amongst members of the *Beddomeia* genus and compares these to additional Hydrobiidae genera including *Austropyrgus*, *Phrantela*, *Nanocochlea*, *Pseudotricula* and the introduced *Potamopyrgus antipodarum*. The morphotypes described in Chapter 5 are included and the findings are reviewed in light of the phylogenetic analyses conducted. The results of these chapters are used to assist with reviewing the taxonomy of *Beddomeia* and provide supporting evidence to the argument for suggested conservation management approaches in the next section.

Beddomeia morphotypes 1 and 2 (left and right respectively) on *Dicksonia antarctica* frond.

Chapter 5

Morphological descriptions of *Beddomeia* morphotypes

Chapter 5 investigates the morphological taxonomy of morphotypes discovered in studies undertaken for this thesis. Eight morphotypes were identified from sites across two catchments in northern Tasmania, some of which are confirmed as *Beddomeia tasmanica* and others closely related to *B. wilmotensis*, *B. inflata* and *B. hallae*. This chapter provides descriptions of the morphotypes which are later included in the DNA analyses in Chapter 6.

Morphotype 2, Groom River catchment

Morphotype C, Castra Rivulet catchment

5 Morphological descriptions of *Beddomeia* morphotypes

5.1 Introduction

Tasmania is recognised as an important area of species diversification within its Hydrobiidae fauna. Despite having a relatively low number of recognised native genera, the Tasmanian hydrobiids include 105 named species and subspecies in the five freshwater genera (Ponder *et al.* 1993, Clark *et al.* 2003, Ponder *et al.* 2005). By far the largest radiation occurs within the genus *Beddomeia*, containing 46 named species and subspecies and there are more, currently undescribed species (Ponder *et al.* 1993). Recent molecular techniques have, for the most part, validated the current speciation within a subset of *Beddomeia*, but also suggest a more diverse speciation is occurring at the catchment level (Chapter 6).

Taxonomic history of *Beddomeia*

The taxonomy of the group has been under review for over 150 years; the first species was described as *Littorina paludinella* Reeve, 1857 (Reeve 1857(-1858), Ponder *et al.* 1993). Further species, *Ampullaria* and *Valvata tasmanica*, were described in 1876 and 1877 by Tenison-Woods (Tenison-Woods 1876, 1877); and Johnston described one, *Amnicola launcestonensis* in 1879 (Johnston 1879). Petterd introduced the genus *Beddomeia* in 1889, offering a further six taxa, two of which were varieties of *B. launcestonensis* (*B. minima* and *B. tumida*) (Petterd 1889). No further species were attributed to the genus until an extensive review of the *Beddomeia* „complex' was undertaken in the early 1990s (Ponder *et al* (1993). This seminal work provides a detailed taxonomic review of the genus complex, attributing the taxa to four genera; *Austropyrgus*, *Beddomeia*, *Nanocochlea* and *Phrantela*, and describes 46 *Beddomeia* species, including subspecies and re-described species. A list of the current named species-group taxa is provided in Appendix A. Ponder *et al.* (1993) also suggest that up to 20 more species may be possible from their work, although, due to limited morphological differences these species were not described. No further taxonomic assessment of the genus has been undertaken.

Identification of *Beddomeia* species remains challenging. Morphological convergence within the Hydrobiidae and closely related families has meant that traits such as shell characteristics, which are said to be „plastic' due to the variability between and within species, are not good indicators of speciation (Ponder *et al.* 1993, Hershler and Ponder 1998). Due to the small average size of species within *Beddomeia* complex, and the convergent nature of family, and

some species sharing similar shell characteristics, field identification below genus level is difficult. To distinguish between hydrobiid species, anatomical characters as well as shell morphology have been utilised (Hershler and Ponder 1998, Miller *et al.* 1999, e.g. Clark *et al.* 2003, Hershler and Liu 2004) and are consistent with the characters used by Ponder *et al*. (1993) to review *Beddomeia*.

Samples taken from Castra Rivulet in northern Tasmania and from Groom River in north-eastern Tasmania (Chapter 3) suggested the presence of several species. In this Chapter a subset of the recognised morphological characteristics (Ponder *et al.* 1993, Hershler and Ponder 1998) is utilised to describe eight *Beddomeia* morphotypes from the two study catchments. It was not intended to produce complete morphological descriptions of these morphs in this paper, but to determine whether sufficient morphological differences exist to support the number of anatomical recognised from shell characters in Chapter 3, and morphometric analyses of shell characters were not conducted.

5.2 Materials and methods

Specimens examined

Specimens were obtained from samples collected in the Castra Rivulet and Groom River catchments between September 2001 and November 2003 as part of a catchment-wide distributional study (Chapter 3). A list of the collection locations is given in Table 5.1. Locations are provided using the GDA datum (for details see http://www.ga.gov.au/geodesy/datums/gda.jsp).

Morphometrics and meristics

Measurements were taken from SEM photographs of morphotypes and from photographs of a minimum of six adult specimens taken through a Leica MZ75 stereomicroscope, using a graticule to establish scale. Measurements are presented with morphotype descriptions.

Table 5.1. Locations from where specimens were collected (GDA datum). Ca refers to Castra Rivulet catchment; GC to Groom River catchment. The associated number refers to stream number (see Figure 3.1*a, b* for stream locations).

Stream Code	Morphotype*	Grid Reference		Accession Number**		
Ca4	A, B	424032	5423003			
Ca7	**A**, D	427352	5422663	E26196		
Ca10	B, C	424592	5422263			
Ca11	B	424472	5422063			
Ca17	**B, C, D**	424682	5421483	E26197	E26198	E26199
GC1	**1**, 2, **3** and 6	581062	5436333	E26200	E26201	
GC7	1	585712	5435233			
GC8	**2**	585512	5435223	E26202		
GC9	**6**	585472	5435633	E26203		
GC15	1, 2 and 3	586002	5431313			
GC16	6	586602	5432363			
GC18	1, 2	584502	5432883			

* Note: morphotype labelling is consistent with chapters 3 and 6.

** Accession numbers for Collections and Research Facilities of Tasmanian Museum and Art Gallery (TMAG), Winkleigh Place, Rosny

Anatomy

A minimum of six specimens of each morphotype were dissected, three of each sex; additional specimens were examined in cases where all specimens available were of the same sex. Specimens examined were fixed in formalin and later transferred to 80% ethanol. Insufficient specimens were available to examine sexual dimorphism. Pigmentation was consistent across all specimens of each morphotype, and was compared with additional fresh specimens collected for morphotypes A and D, which were preserved in 80% ethanol and dissected within 20 hours of collection.

Dissections were conducted in a small black watch glass under a Leica MZ75 stereomicroscope. Scoring was done in accordance with a subset of characters and measurements described in Ponder *et al.* (1993) and Hershler & Ponder (1998) and are grouped by the following external and internal characters:

Shell: shell length (SL), shell width (SW), length of body whorl (BW), width of body whorl (WB), length of aperture (AL), width of aperture (AW), umbilicus width; periostracum colour and thickness; number of protoconch whorls and microsculpture; teleoconch whorls,

teleosculpture; periphery of last whorl; inner lip thickness and columellar swelling, orientation of edge of outer apertural lip and umbilicus width. The main shell measurements used in identification are represented in Figure 5.1. Diagrams of other anatomical features are not reproduced here, but can be found in (Smith and Kershaw 1991, Ponder *et al.* 1993, Hershler and Ponder 1998).

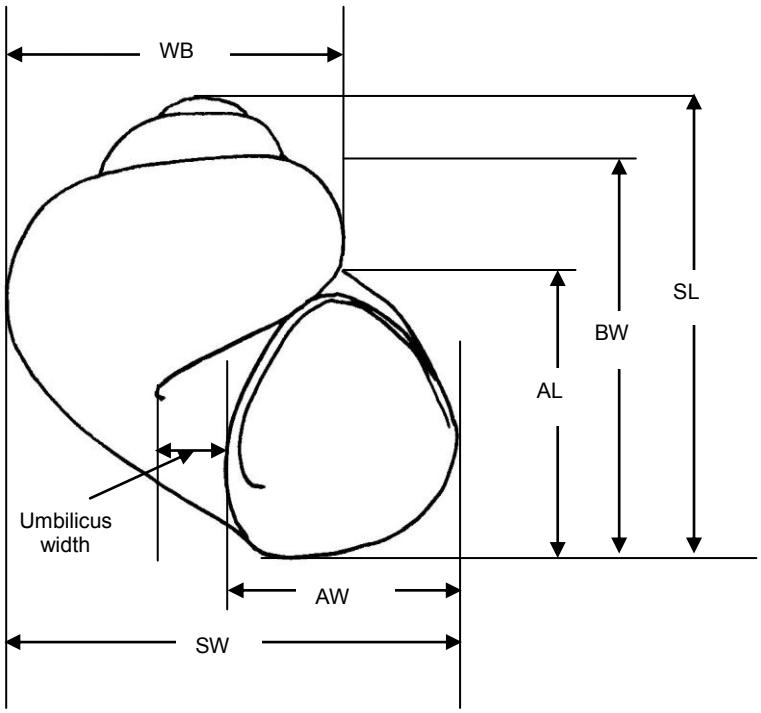


Figure 5.1. Diagram of shell measurements used.

Head-foot: extent of pigmentation on pallial roof, on cephalic tentacles and between cephalic tentacles. Pigmentation was also noted on body whorls.

Non-genital anatomy: pallial tentacle presence; ctenidium width and length, number of ctenidium filaments and location of apices; osphradium shape; hypobranchial gland development; rectum shape and orientation of faecal pellets, anus position; position of renal organ, renal gland shape; size of caecum in relation to stomach.

Male reproductive system: testis length; seminal vesicle location; proportion of prostate gland in pallial roof, shape of prostate and location of vas deferens opening; shape of anterior portion of the vas deferens; penis size, shape and folding.

Female reproductive system: ovary shape and size; bending of proximal coiled oviduct and shape of distal portion; coiled oviduct and bursal duct joining with respect to posterior pallial wall; size, shape and position of bursa copulatrix; seminal receptacle shape and location; capsule gland thickness; ventral channel features and location and size of genital opening.

Shell images

Shells were mounted on aluminium stubs and examined using scanning electron microscope (SEM). The clarity of preliminary images was assessed to be suitable for scanning without requiring samples to be coated with gold. Specimens were cleaned using 15% hypochlorite bleach and brushing with a fine paintbrush to remove adherent material prior to mounting.

5.3 Results

The morphotypes recorded from the two catchments in Chapter 3 were originally identified based on external shell characters: SL, SW, BW and AL. To distinguish between morphotypes from each catchment, Castra morphotypes were designated letters A to D while Groom River morphotypes were numbered 1 to 6 (Figures 5.1*A-D* and 5.3*A-E*); noting that morphs 4 and 5 were from catchments immediately adjacent to the Groom River catchment and were not the focus of the Chapter 3 study, hence are not included here. As discussed in Chapter 3, the morphotypes identified are patchily distributed and abundance was found to differ throughout each river catchment, multiple morphotypes also occurred sympatrically in most streams (Table 5.2).

Table 5.2. Distribution of each morphotype in catchments surveyed, out of a total of 18 streams per catchment. (Also refer to Chapter 3, Table 3.4 and Figures 3.1*a, b*)

Morphotype	A	B	C	D	1	2	3	6
Streams present (No.)	6	12	7	14	14	12	13	17

Detailed descriptions and diagrams explaining each of the characters used in the following morphotype descriptions are presented in Ponder *et al.* (1993) and Hershler & Ponder (1998) and should be consulted for explanation of some terms used below. A comparative summary table of the key morphological differences between each morphotype is presented in Table 5.11.

5.3.1 Description of morphotypes from the Groom River catchment

Morphotype 1

(Figure 5.2*A-B*, 5.6*A-B*, 5.7*A-B*, 5.8*A*)

Material Examined

From GC7, first order stream, tributary of Groom River, on Anchor Creek Rd, northern Tas., 585712 5435233 GDA. *Additional material*. GC15, first order stream, tributary of Groom River at junction of Anchor Creek Rd and Tasman Hwy near Pyengana, 586002 5431313, GC18, second order stream, tributary of Groom River, off Lehners Ridge Rd, 584502 5432883, and GC1, first order stream, tributary of Dead Horse Hill Creek, tributary of Groom River, on Lottah Rd, northern Tas., 581062 5436333.

Diagnosis

Differences from other members of the Groom River group are limited to shell proportions, the number of ctenidial filaments, the degree of arching in the rectum, the testis whorls and the presence of a penis with long tapering distal end.

Description

Shell. (Figs 5.2*A-B*). Trochiform, 1.89-1.98 mm in length, 1.79-2.0 mm in width. SW/SL 0.94-1.01, AL/SL 0.53-0.59. Periostracum yellow to darkly pigmented. Protoconch 1.4-1.5 whorls; microsculpture uniform, with faint, spirally arranged wrinkles. Teleoconch of 2.7-3.0 convex whorls, sculpture of faint, prosocline growth lines (although several specimens (< 0.05%) showed strong, prominent prosocline growth lines); periphery of last whorl evenly rounded. Inner lip thin to medium thickness and width, columellar swelling absent. Outer lip prosocline. Umbilicus wide, 0.3-0.35 mm in width.

Head-foot. Pigmentation on whorls posterior to body whorl and medial longitudinal streak on cephalic tentacles. Pallial roof pigmentation uniform, with dark pigmentation between cephalic tentacles.

Non-genital anatomy. Pallial tentacle absent. Ctenidium narrow to moderately wide, extending almost entire length of pallial cavity, 16-18 filaments, apices on right. Osphradium elongate, medium width, wider at anterior end, anterior end simple. Osphradium opposite posterior part of ctenidium. Hypobranchial gland moderately developed, with smooth surface. Rectum moderately arched, with faecal pellets obliquely to longitudinally arranged. Anus intermediate

in position relative to mantle edge (for details refer to Figure 4, pg 510, Ponder *et al*. 1993). Renal organ not extending forward into pallial roof, long axis variable. Stomach with small caecum.

Table 5.3. Shell dimensions of morphotype 1.

Shell dimensions	AW	SW	SL	WB	BW	AL
	1.01	2.00	1.96	1.44	1.70	1.16
	1.03	1.79	1.89	1.45	1.67	1.00
	1.02	1.85	1.91	1.43	1.68	1.08
	1.05	1.95	1.94	1.46	1.69	1.17
	1.01	1.96	1.97	1.41	1.66	1.07
	1.02	1.87	1.98	1.44	1.69	1.04
Mean	**1.02**	**1.90**	**1.94**	**1.44**	**1.68**	**1.09**

* Note: shell measurement in table are for unsexed individuals

Male reproductive system. (Figs 5.7*A*-*B*, 5.8*A*). Testis of 1.5-2.0 whorls. Seminal vesicle beneath less than anterior 1/4 of first whorl of testis behind stomach; coiled over stomach. Prostate gland occupying 1/2 of pallial roof, elongate pyriform, anterior end simple, oval in profile; vas deferens opening anteriorly. Pallial vas deferens coiled posteriorly. Penis medium-sized relative to head (refer to Figure 20, pg 47, Hershler and Ponder 1998), with distal end long, tapering, blunt, without papilla; medial section tapering, with weak to moderate folds, penial duct straight to weakly undulating in medial and basal sections; base wide, with moderate folds.

Female reproductive system. (Figs 5.6*A*-*B*). Ovary simple, occupying about 0.3-0.5 whorls. Proximal coiled oviduct with 2 bends, initial U bend orientated dorso-ventrally; bound in connective tissue, of soft, glandular appearance, extending to posterior edge of bursa copulatrix; distal portion 1 bend. Coiled oviduct and bursal duct joining in front of posterior pallial wall at posterior pallial wall. Bursa copulatrix small to medium, not extending to posterior pallial wall, globular. Bursal duct arising from anterior edge of bursa. Seminal receptacle pyriform, at ventral to postero-ventral edge of bursa copulatrix. 2/3 of albumen gland anterior to posterior wall. Capsule gland swollen, about 2/3 length of albumen gland. Ventral channel with anterior vestibule. Genital opening terminal, small.

Remarks

Morphotype 1 closely resembles *B. tasmanica* and is likely to be a variant of this species (refer to Ponder *et al*. 1993, pp 542-545). The morph superficially shares similar shell characters with the Type specimen of *B. tasmanica*, but SL and SW are larger, the AL/SL ratio is smaller (0.62-0.66 for *B. tasmanica* compared to 0.53-0.59) and the number of teleoconch whorls differ. However, in comparison to the additional material obtained by Ponder *et al.* (1993) these measures fit within the described ranges for *B. tasmanica.* The number of protoconch whorls also fit within the ranges for the Type and addition material (1.3-1.67), although there are minor microsculpture differences. Pigmentation often fades with preservation, so it is not considered unusual for the specimens obtained for this study to show dark body pigmentation while the original descriptions indicate none existed. Reproductive characters are similar to those described for *B. tasmanica*, the only exception is that the range of whorls occupied by the ovary is slightly lower in morph 1 (0.3-0.5 compared to 0.4-0.6 whorls). One significant difference between this morph and *B. tasmanica* is the number of bends in the proximal coiled oviduct (2 bends in morph 1 compared to a single bend in *B. tasmanica*).

Morphotype 2

(Figure 5.2*C*, 5.6*C*, 5.7*C-D*, 5.8*B-C*)

Material Examined

From GC8, first order stream, tributary of Groom River, northeast Tas., 585512 5435223 GDA. *Additional material*. GC15, first order stream, tributary of Groom River at junction of Anchor Creek Rd and Tasman Hwy near Pyengana, 586002 5431313. GC18, second order stream, tributary of Groom River, off Lehners Ridge Rd, 584502 5432883.

Diagnosis

Differs from other members of the Groom River group in having the most depressed shell, widest aperture, and reduced number of teleoconch whorls, also in the coiling of the proximal coiled oviduct, prostate shape and undulating nature of the pallial vas deferens.

Description

Shell. (Fig 5.2*C*). Depressed trochiform, 1.66-1.74 mm in length, 1.80-1.91 mm in width. SW/SL 1.08-1.10, AL/SL 0.66-0.67. Periostracum pale yellow to light brown. Protoconch about 1.5 whorls; microsculpture uniform, smooth to very faint, spirally arranged wrinkles. Teleoconch of 2.2-2.6 convex whorls, sculpture of faint, prosocline growth lines; periphery of

last whorl evenly rounded. Inner lip thin to medium thickness and width, columellar swelling absent. Outer lip prosocline. Umbilicus wide, 0.3-0.35 mm in width.

Head-foot. Pigmentation on whorls posterior to body whorl, medial longitudinal streak on cephalic tentacles; pallial roof pigmentation uniform, dark melanic pigmentation between cephalic tentacles.

Table 5.4. Shell dimensions of morphotype 2.

Shell dimensions	AW	SW	SL	WB	BW	AL
	1.04	1.89	1.67	1.36	1.55	1.16
	1.03	1.80	1.74	1.36	1.68	1.15
	1.02	1.83	1.70	1.35	1.62	1.14
	1.05	1.91	1.69	1.37	1.62	1.16
	1.07	1.88	1.71	1.38	1.58	1.12
	1.01	1.80	1.66	1.35	1.56	1.11
Mean	**1.04**	**1.85**	**1.69**	**1.36**	**1.60**	**1.14**

* Note: shell measurement in table are for unsexed individuals

Non-genital anatomy. Pallial tentacle absent. Ctenidium narrow, extending most of length of pallial cavity; 14-19 filaments, apices on right. Osphradium elongate, medium width, wider at anterior end, anterior end simple. Hypobranchial gland weakly developed, with smooth surface. Rectum slightly to moderately arched, with faecal pellets obliquely arranged. Anus position intermediate (refer to Figure 4, Ponder *et al*. 1993). Renal organ not extending forward into pallial roof, long axis variable. Stomach with small caecum.

Male reproductive system. (Figs 5.7*C-D*, 5.8*B-C*). Testis of 1.5-1.8 whorls. Seminal vesicle beneath less than anterior 1/4 of first whorl of testis behind stomach; coiled over stomach. Prostate gland occupying1/2 to 2/3 of pallial roof, pyriform to banana-shaped, anterior end simple, oval in profile; vas deferens opening anteriorly. Pallial vas deferens undulating posteriorly. Penis medium-sized, with distal end moderate, tapering, blunt, without papilla; medial section tapering, with moderate folds, penial duct straight in medial and basal sections; base moderate, with moderate to strong folds.

Female reproductive system.(Fig 5.6*C*). Ovary simple, occupying about 0.5 whorls. Proximal coiled oviduct with 2 bends, U-shaped initial bend orientated dorso-ventrally to slightly posteriorly oblique; bound in connective tissue, of soft, glandular appearance, extending

posterior to bursa copulatrix; distal portion straight. Coiled oviduct and bursal duct joining at, or immediately in front of posterior pallial wall. Bursa copulatrix of medium size, not extending to posterior pallial wall; globular. Bursal duct arising from anterior edge of bursa. Seminal receptacle pyriform, at ventral to postero-ventral edge of bursa copulatrix. 2/3 of albumen gland in front of posterior wall. Capsule gland swollen, about 2/3 length of albumen gland. Ventral channel with anterior vestibule. Genital opening terminal, small.

Remarks

The shell of morphotype 2 is more depressed and has a wider aperture than morph 1. While the shell measurements fall within the ranges described for *B. tasmanica*, the SW/SL ratio is larger for this morphotype (SW/SL of 1.08-1.10 compared to 0.87-1.03) and the AL/SL ratio is at the upper end of the *B. tasmanica* range (0.66-0.67 compared to 0.53-0.67). As with morphotype 1, this morph displays many anatomical characters in common with *B. tasmanica*, but differs in the development of the hypobranchial gland (weak in morph 2 compared with thick to moderately developed), presence of an undulating vas deferens in the mid to basal sections of the penis, and strong penial folds. As with morph 1, one difference between this morph and *B. tasmanica* is the number of bends in the proximal and distal sections of the coiled oviduct (2 proximal bends and straight distal section in morph 2 compared to a single bend in proximal and distal sections in *B. tasmanica*).

Morphotype 3

(Figure 5.2, 5.7*F*, 5.8*D*)

Material Examined

From GC1, first order stream, tributary of Dead Horse Hill Creek, tributary of Groom River, on Lottah Rd, northern Tas., 581062 5436333. *Additional material*. GC15, first order stream, tributary of Groom River at junction of Anchor Creek Rd and Tasman Hwy near Pyengana, 586002 5431313.

Diagnosis

Differs from other members of the Groom River group in shell shape, the separation of last whorl, having a closed umbilicus, a wide ctenidium, strong to double arched rectum and the position of the anus (all others intermediate position), also the shape of the prostate in cross section. *Only males recorded.*

Description

Shell. (Fig 5.2*D*). Broadly conic, with separation of last whorl, 1.74-1.81 mm in length; 1.41-1.42 mm in width. SW/SL 0.78-0.81, AL/SL 0.51-0.55. Periostracum pale yellow to dark brown. Protoconch of 1.3-1.4 whorls; smooth. Teleoconch of 2.8-3.0 whorls. Teleoconch sculpture of faint, prosocline growth lines, periphery of last whorl evenly rounded. Inner lip thin, narrow, columellar swelling absent. Outer lip prosocline. Umbilicus small to closed (< 0.1 mm wide).

Table 5.5. Shell dimensions of morphotype 3.

Shell dimensions	AW	SW	SL	WB	BW	AL
	0.81	1.42	1.81	1.26	1.58	0.99
	0.86	1.41	1.74	1.24	1.50	0.89
	0.87	1.42	1.80	1.25	1.53	0.92
	0.85	1.41	1.75	1.24	1.52	0.91
	0.82	1.42	1.79	1.25	1.55	0.97
	0.83	1.41	1.78	1.24	1.51	0.92
Mean	**0.84**	**1.415**	**1.78**	**1.25**	**1.53**	**0.93**

* Note: shell measurement in table are for unsexed individuals

Head-foot. Pigmentation on whorls posterior to body whorl, longitudinal streaks on cephalic tentacles, and uniform pallial roof pigmentation on posterior 2/3 between cephalic tentacles.

Non-genital anatomy. Pallial tentacle absent. Ctenidium wide (broader than high), extending almost entire length of pallial cavity, 15-19 filaments, apices on right. Osphradium elongate, of medium width, slightly wider at anterior end, anterior end simple. Hypobranchial gland weakly developed, with smooth surface. Rectum strongly arched (double arch in two specimens), faecal pellets longitudinally to obliquely oriented. Anus at pallial edge. Renal organ not extending forward into pallial roof, renal gland thin, orientated with long axis longitudinal. Stomach with small to very small caecum.

Male reproductive system. (Figs 5.7*F*, 5.8*D*). Testis of 1 to 1.4 whorls. Seminal vesicle beneath less than anterior 1/4 of first whorl of testis behind stomach; coiled over stomach. Prostate gland occupying about 1/3 of pallial roof, pyriform, compressed in section, closed; vas deferens opening anteriorly. Pallial vas deferens straight. Penis small to moderate-sized; broadly triangular, with distal end tapering, blunt, without papilla; medial section tapering, with

moderate to strong folds, penial duct weakly undulating in medial and basal sections; base moderate, with moderate folds.

Remarks

No females were recorded for this morphotype from the 14 dissections conducted on specimens from two streams. While sexual dimorphism with other morphs cannot be fully discounted, it is more likely that females of this morph exist, as morph 3 differs substantially in shell characters from the other morphotypes in the Groom River catchment, being broadly conic in shape, possessing a separated last whorl and having a small to closed umbilicus. The shell of this species is intermediate between *B. briansmithi* and *B. fromensis*, but differs from both having a separation of the last whorl. Morphotype 3 shares some anatomical characters in common with *B. briansmithi*, although not all. While this morph is smaller in shell length (1.77-1.87 mm) than *B. briansmithi* (up to 2.11 mm), the SW/SL and AL/SL ratios are similar. Other differences between morph 3, *B. fromensis* and *B. briansmithi* include: the number of teleoconch whorls (more in morph 3), umbilicus size, osphradium size and shape, position of anus and penis size.

Morphotype 6

(Figure 5.2*E*, 5.6*D*, 5.7*G-H*, 5.8*E-F*)

Material Examined.

From GC9, first order stream, tributary of Groom River, on Anchor Creek Rd, northern Tas., 585472 5435633. *Additional material*. GC16, second order stream, tributary of Groom River, on Anchor Creek Rd, northern Tas., 586602 5432363.

Diagnosis

Differs from other members of the Groom River group (morphs 1 and 2) in osphradium width, the weak pitting on protoconch, and having more variability in the ctenidium.

Description

Shell. (Figure 5.2*E*). Trochiform to depressed trochiform, 1.77-1.87 mm in length, 1.66-1.85 mm in width. SW/SL 0.94-0.99, AL/SL 0.63-0.64. Periostracum light yellow to dark brown pigmented. Protoconch between 1.4-1.5 whorls; microsculpture uniform, very weak pitting and very faint, spirally arranged wrinkles. Teleoconch of 3.0-3.2 convex whorls, sculpture of faint, prosocline growth lines; periphery of last whorl evenly rounded. Inner lip thin to medium thickness and width, columellar swelling absent. Outer lip prosocline. Umbilicus wide, 0.32-0.35 mm in width.

Head-foot. Pigmentation behind and across eyes. Pigmentation on whorls posterior to body whorl, on cephalic tentacles and on pallial roof between cephalic tentacles. Tentacle with medial longitudinal streak. Pallial roof pigmentation uniform.

Non-genital anatomy. Pallial tentacle absent. Ctenidium narrow, extending almost entire length of pallial cavity, 11-18 filaments, apices on right. Osphradium medium width, wider at anterior end, anterior end simple. Hypobranchial gland weakly to moderately developed, with smooth surface. Rectum slight to moderately arched, with faecal pellets longitudinally arranged. Anus intermediate in position. Renal organ not extending forward into pallial roof. Stomach with small caecum.

Table 5.6. Shell dimensions of morphotype 6.

Shell dimensions	AW	SW	SL	WB	BW	AL
	0.98	1.66	1.77	1.33	1.58	1.11
	0.96	1.85	1.87	1.36	1.66	1.20
	0.97	1.75	1.82	1.34	1.60	1.17
	0.96	1.78	1.83	1.36	1.62	1.16
	0.94	1.82	1.79	1.32	1.59	1.16
	0.94	1.77	1.80	1.33	1.60	1.14
Mean	**0.96**	**1.77**	**1.81**	**1.34**	**1.61**	**1.16**

* Note: shell measurement in table are for unsexed individuals

Male reproductive system. (Fig 5.7*G-H*, 5.8*E-F*). Testis of 1.5-1.9 whorls. Seminal vesicle beneath less than anterior 1/4 to 1/3 of first whorl of testis behind stomach; coiled over stomach. Prostate gland occupying about 1/2 of pallial roof, pyriform, broadly oval to circular in section; vas deferens opening anteriorly. Pallial vas deferens straight. Penis medium-size, with distal end long, tapering, simple, without papilla; medial section tapering, penial duct straight in medial and basal sections; base moderate to wide, with weak to moderate folds.

Female reproductive system.(Fig 5.6*D*). Ovary simple, occupying about 0.4-0.5 whorls. Proximal coiled oviduct with 3 bends, with initial U bend orientated obliquely backwards, and subsequent bends at right angles; bound in connective tissue, of soft, glandular appearance, not extending to posterior edge of bursa copulatrix; distal portion straight to seminal receptacle. Coiled oviduct and bursal duct joining in front of posterior pallial wall at posterior pallial wall. Bursa copulatrix of small to medium size; posteroventrally positioned, not extending to posterior pallial wall; globular. Bursal duct arising from anterior edge of bursa. Seminal

receptacle pyriform, at ventral to postero-ventral edge of bursa copulatrix (disassociated from bursa in Figure 5.6*D*). 2/3 of albumen gland in front of posterior wall. Capsule gland swollen, about 2/3 length of albumen gland. Ventral channel with anterior vestibule. Genital opening terminal, small.

Remarks

Together with morphotypes 1 and 2, most anatomical characters of morph 6 are similar to those of *B. tasmanica*. Once again, the major difference is in the coiling of the proximal oviduct, but also in the penis size (smaller than other morphs). The number of bends in the proximal and distal sections of the coiled oviduct differ from *B. tasmanica* (3 bends forming 2 proximal loops at right angles to each other in the proximal section and straight distal section in morph 3).

5.3.2 Remarks on Groom River group

Based on shell characters, morphotype 3 is distinctly different from the others, being broadly conical, having a thin inner lip, and its aperture shape showing separation of the last whorl. The remaining three morphotypes are more similar in shell morphology (trochiform to depressed trochiform) and in pigmentation. Differences in the latter three morphs include: the number of teleoconch whorls, the number of ctenidium filaments, the development of the hypobranchial gland, prostate shape and penis characteristics. Pigmentation differed only in intensity between the four morphotypes. No females were recovered for morphotype 3, despite dissection of a further eight specimens. It remains probable that the females exist, but were not recovered during the original sampling, particularly as this morphotype occurred infrequently and was the least abundant of the Groom River morphotypes. This possibility is further supported by a similar observation originally made for morphotype 6, where only males were recorded from eight dissections; however, upon subsequent sampling and dissections only females were identified from ten specimens taken from a nearby location.

Egg capsules recovered from the Groom River sites were conspicuous on darker material such as *Dicksonia antarctica* fronds and woody debris, but blended in on the lighter granite substrate; some were constructed on granite gravel. Capsules were spherical to ovoid, dome-shaped to flat topped, with a broad attachment base and were covered with fine grains of granite sand; they were 0.6-0.95 mm in maximum length and contained single eggs (Figure 5.3*a, b*). Of the estimated 30 egg capsules examined, each was constructed of a similar granite sand; none contained wood fragments. The egg capsules of *B. tasmanica* are unknown, but those of some other northeast Tasmanian species have been documented in Ponder *et al.* (1993), those reported

in this study are typically of similar size, although some are smaller than any previously recorded.

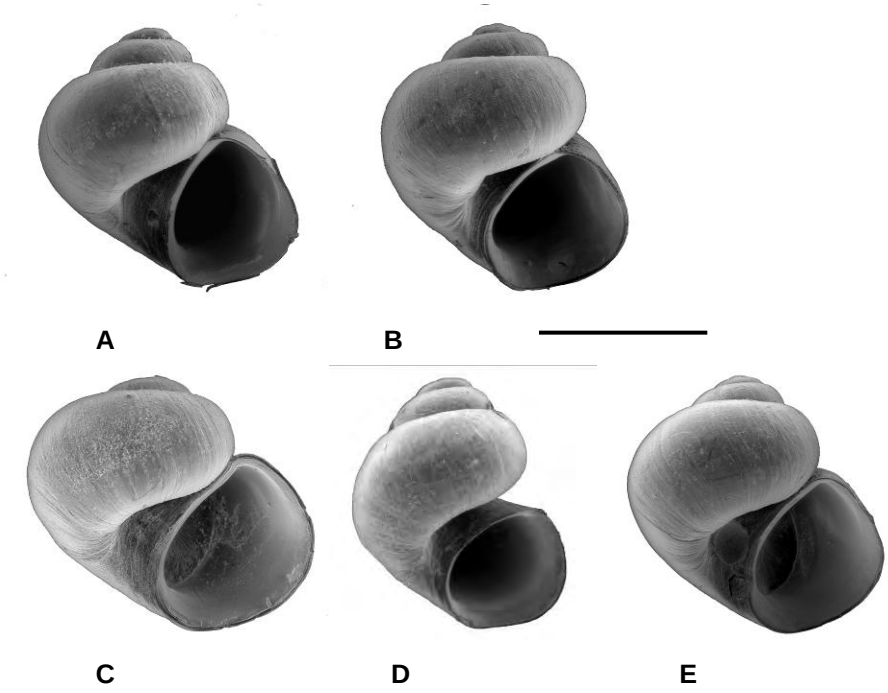
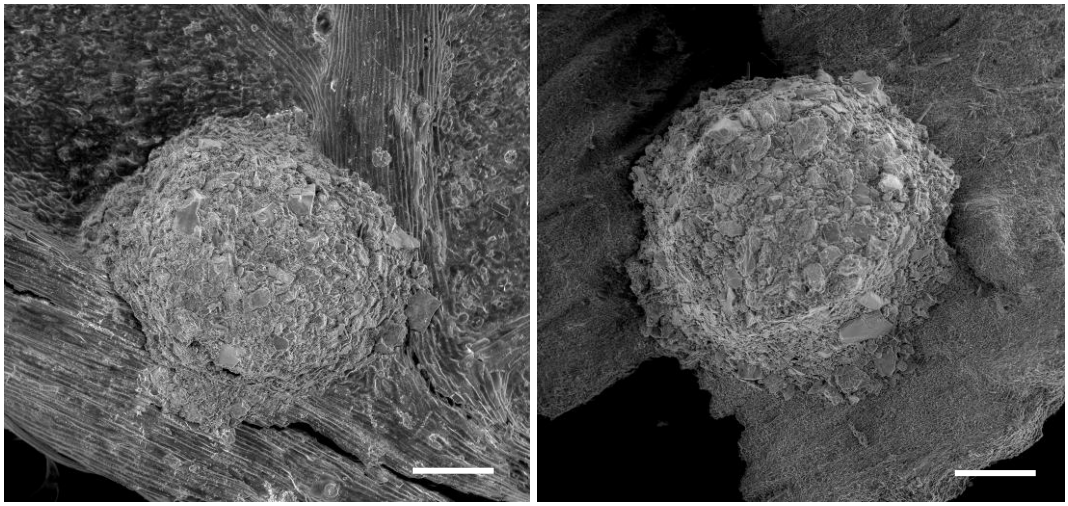


Figure 5.2. Shells of species of *Beddomeia* from Groom River catchment: ***A-B***, morphotype 1; ***C***, morphotype 2; ***D***, morphotype 3; ***E***, morphotype 6. Scale bar: 1 mm.



Figures 5.3*a* and *b*. Groom River catchment – 5.3*a*, *Beddomeia* sp. egg capsule at junction of pinnule on *Dicksonia antarctica*, 5.3*b*, *Beddomeia* egg capsule on single grain of granite, Scale 200 µm.

5.3.3 Description of morphotypes from the Castra Rivulet catchment

Morphotype A

(Figure 5.4*A*, 5.6*E*, 5.7*I-J*, 5.8*G*)

Material Examined

From Ca7, first order stream, tributary of Deep Gully Creek, on Ghost Hole Rd, Upper Castra, northern Tas., 427352 5422663 GDA, 350 m, 2004. Snails present on leaves and woody material. *Additional material.* Ca11, first order stream off Flints Road, Upper Castra, 424472 5422063; Ca4, first order stream off unnamed track off Castra Rd, Upper Castra, 424032 5423003.

Diagnosis

Differs from other members of the Castra group in its ovate shell shape, the presence of wrinkles on protoconch, thin inner lip; head and body unpigmented; and fewer, broad ctenidium filaments (12 – 15) with central apices; long S-shape folds in rectum; testis restricted to 1 whorl, blunt distal end on penis.

Description

Shell. (Fig 5.4*A*). Ovate, 2.96-4.20 mm in length; 2.29-3.45 mm in width. SW/SL 0.77-0.82, AL/SL 0.54-0.59. Periostracum off white to pale yellow. Protoconch of about 1.5 whorls; microsculpture uniform, weakly pitted, with faint spirally arranged wrinkles. Teleoconch of 3.0-3.5 convex whorls, teleosculpture faint, prosocline growth rings; periphery of last whorl evenly rounded. Inner lip thin, narrow, columellar swelling absent. Outer lip prosocline. Umbilicus small (closed to 0.2 mm wide).

Head-foot. Eyes, head and body unpigmented (confirmed with fresh specimens).

Non-genital anatomy. Pallial tentacle absent (present on one specimen). Ctenidium intermediate, occupying almost entire length of pallial cavity (and onto mantle in one specimen), 12-15 filaments, broader than high, or as broad, with central apices. Osphradium small, relative to the length of the pallial cavity (< 50% of ctenidium length), opposite anterior part of ctenidium, oval to elongate, medium width, anterior end simple. Hypobranchial gland thick, smooth surface. Rectum long S-shape, with faecal pellets oblique to variably arranged in some specimens. Anus near pallial edge (for detailed explanation refer to Figure 4 in Ponder *et*

al. 1993). Renal organ not extending forward into pallial roof, renal gland circular. Stomach with small caecum.

Table 5.7. Shell dimensions of morphotype A.

Shell dimensions	AW	SW	SL	WB	BW	AL
	1.94	3.45	4.20	2.80	3.45	2.26
	2.05	3.34	4.20	2.91	3.66	2.26
	1.83	3.12	3.98	2.69	3.23	2.15
	1.72	3.18	4.09	2.58	3.12	2.15
	1.72	3.12	3.77	2.58	3.02	2.05
	1.72	2.91	3.50	2.53	2.91	2.05
	1.83	3.34	3.98	2.69	3.34	2.26
Mean	**1.83**	**3.21**	**3.96**	**2.68**	**3.25**	**2.17**

* Note: shell measurement in table are for unsexed individuals

Male reproductive system.(Figs 5.7*I-J*, 5.8*G*). Testis of about 1 whorl. Seminal vesicle beneath less than anterior 1/4 of first whorl of testis behind stomach; not conspicuously coiled over stomach. Prostate gland occupying about 1/3 to 1/2 of pallial roof, oval to elongate, compressed in section, short posterior opening; vas deferens opening anteriorly. Pallial vas deferens straight. Penis medium-sized, with distal end tapering (with blunt tip), simple, without papilla; medial section parallel sided, of medium length, penial duct straight to weakly undulating in medial and basal sections; base moderately wide. Weak folds in medial to distal section only.

Female reproductive system.(Fig 5.6*E*). Ovary simple to lobulate, occupying about 0.4 whorls. Proximal coiled oviduct with 2 bends on most specimens (one specimen with 3 bends), with initial U-shape bend orientated dorso-ventrally; bound in connective tissue, of soft, glandular appearance; extending to posterior of bursa copulatrix; proximal part inverted U-shape, distal portion straight. Coiled oviduct and bursal duct join at posterior pallial wall. Bursa copulatrix of small to medium size, not extending to posterior wall, globular. Bursal duct arising from anterior edge of the bursa. Seminal receptacle pyriform, between posterior and ventral edge of bursa copulatrix. More than 2/3 of albumen gland in front of posterior wall. Capsule gland thin, about 2/3 length of albumen gland. Ventral channel with anterior vestibule. Genital opening terminal, small.

Remarks

Dimensions of morphotype A are similar to both *B. wilmotensis* and *B. inflata* (Ponder *et al.* 1993), but overall shell length is more comparable to *B. inflata.* The number of ctenidial filaments of morphotype A are low, 12-15 filaments, and in line with those reported for *B. wilmotensis*, and the lobulate nature of the ovary of morph A is also a feature common to *B. wilmotensis.* However, morphotype A differs from *B. wilmotensis* in a number of characters including possessing a well developed hypobranchial gland (in line with *B. inflata*), length of rectum (longer than either species), shape of prostate (similar to *B. inflata*) and number of bends in the proximal coiled oviduct (two versus three bends, again, similar to *B. inflata*).

Large specimens (SL > 3.0 mm) were rarely encountered in the catchment study (Chapter 3), being confined to two streams surveyed in the Castra catchment, Ca 7 and Ca 8 and an unmarked tributary of Ca 7. Where present, most streams contained specimens SL < 3.0 mm (average SL = 2.87 mm), although the largest of these showed adult characteristics including thickening of outer lip and well formed reproductive organs.

This morphotype lives beneath basalt stones and on the underside of coarse particulate woody debris (CPOM), typically musk (*Olearia argophylla*), dogwood (*Pomaderris apetala*) and eucalypt (*Eucalyptus obliqua* and *E. regnans*) leaves and branches. It was found living sympatrically with two species of *Austropyrgus*, one of which was *A. lochi*, and three other *Beddomeia* morphotypes.

Morphotype B

(Figure 5.4*B*, 5.6*G-H*, 5.7*L-M*, 5.8*I*)

Material Examined

From Ca 10, second order stream, tributary of Deep Gully Creek, off Flints Rd, Castra, northern Tas., 424592 5422263 GDA. *Additional material*. Ca 11, first order stream off Flints Road, Upper Castra, 424472 5422063; Ca 4, first order stream off unnamed track off Castra Rd, Upper Castra, 424032 5423003.

Diagnosis

Differs from other members of the Castra group in the number of ctenidium filaments, osphradium shape, rectum shape and the number of testis whorls. Differences include the number of bends in the proximal coiled oviduct and the presence of a brown glandular lobe on the convex edge of the penis.

Description

Shell. (Fig 5.4*B*). Conical, 2.72-2.83 mm in length; 1.71-1.77 mm in width. SW/SL 0.62-0.63, AL/SL 0.47-0.49. Periostracum thin, colourless to yellow pigmentation. Protoconch of about 1.5 whorls; microsculpture uniform, smooth, with faint spirally arranged wrinkles. Teleoconch of 3.5 convex whorls; with faint prosocline growth sculptures. Periphery of last whorl evenly rounded. Inner lip of medium thickness and width, columellar swelling absent. Outer lip prosocline. Umbilicus small to closed (0.1 mm wide).

Table 5.8. Shell dimensions of morphotype B.

Shell dimensions	AW	SW	SL	WB	BW	AL
	1.06	1.72	2.76	1.54	2.04	1.34
	1.07	1.74	2.79	1.52	2.00	1.28
	1.08	1.73	2.72	1.57	2.06	1.38
	1.09	1.77	2.83	1.58	2.08	1.36
	1.05	1.71	2.80	1.53	1.98	1.29
	1.06	1.74	2.75	1.57	2.01	1.30
Mean	**1.07**	**1.74**	**2.78**	**1.55**	**2.03**	**1.33**

* Note: shell measurement in table are for unsexed individuals

Head-foot. Eyes and head unpigmented. Dark melanic (grey-brown) speckled pigmentation on dorsal side of final two whorls (posterior to body whorl).

Non-genital anatomy. Pallial tentacle absent. Ctenidium wide, extending almost entire length of pallial cavity, 18-22 filaments, apices on right. Osphradium medium width, oval to elongate, anterior end simple. Hypobranchial gland well developed, ridged. Rectum displaying a prominent arch, faecal pellets arranged obliquely. Anus near pallial edge. Renal organ extending forward less than 1/2 length of renal gland into pallial roof, renal gland orientation variable. Stomach with small caecum.

Male reproductive system. (Figs 5.7*L-M*, 5.8*I*). Testis of 1.5 whorls. Seminal vesicle beneath more than anterior 1/4 of first whorl of testis behind stomach; coiled over stomach and forming large, tightly coiled mass behind stomach. Prostate gland occupying 2/3 of pallial roof, elongate pyriform, broadly oval to circular in section, with thin ventral wall; vas deferens opening anteriorly. Pallial vas deferens straight. Penis medium-sized, with distal end tapering, without papilla; medial section tapering, of medium length, with weakly undulating penial duct in

medial and basal sections; with brown glandular lobe on convex edge of basal section; base moderately wide, with weak to moderate folds.

Female reproductive system. (Fig 5.6*G-H*). Ovary simple, occupies about 0.4-0.5 whorls. Proximal coiled oviduct with 3 bends, with initial U bend orientated obliquely backwards; bound in connective tissue, of soft, glandular appearance, extending to posterior edge of bursa copulatrix; curved distal to seminal receptacle. Coiled oviduct and bursal duct joining in front of posterior pallial wall at about 1/4 from posterior pallial wall to posterior edge of capsule gland. Bursa copulatrix moderate, not extending to posterior wall; globular. Bursal duct arising from ventral edge of the bursa. Seminal receptacle pyriform, between posterior and ventral edge of bursa copulatrix on most specimens (Fig 5.5*H*). Half to 2/3 of albumen gland in front of posterior wall. Capsule gland intermediate thickness, about 2/3 length of albumen gland. Ventral channel with anterior vestibule. Genital opening anterior to capsule gland, small.

Remarks

Shell characters of morphotype B show similarities to *B. hallae* and *B. fallax*, although the shell length of the morph is smaller, the SW/SL and AL/SL ratios of morph B fall within the ranges for both these species. Protoconch whorls are similar to *B. fallax*; the teleoconch whorls are slightly higher than for *B. fallax* (3.5 compared with 3.25-3.4) but similar to the upper value for *B. hallae*. The ctenidium is wide, as is the case in *B. fallax*, while the number of ctenidial filaments, 18-22, falls between those recorded for the two species. Differences are observed in the reproductive organs, with the number of bends in the coiled oviduct of morph B greater than for either species, while the penis displays a prominent brown glandular lobe on the convex edge of its basal section, which has not been detected on the two named species. This morphotype occurs sympatrically with morph D and two *Austropyrgus* species in most streams within the Castra Rivulet catchment (Chapter 3), and like morph D, is abundant, occurring in high numbers in many streams.

Morphotype C

(Figure 5.4*C*, 5.6*F*, 5.7*K*, 5.8*H*)

Material Examined

From Ca10, second order stream, tributary of Castra Rivulet, off Flints Rd, Nietta, northern Tas., 424592 5422263 GDA. *Additional material*. Ca17, first order stream off Flints Rd, Nietta, 424682 5421483.

Diagnosis

Differs from morphotypes B and D of the Castra group in the extension of the shell aperture and oval shape, having the smallest number of ctenidial filaments, a ctenidium extension in the pallial roof, hypobranchial gland development, the extension of renal gland, the coiling of proximal coiled oviduct and the number of testis whorls.

Description

Shell. (Fig 5.4*C*). Conical, 2.39-2.48 mm in length; 1.58-1.64 mm in width. SW/SL 0.66, AL/SL 0.48-0.49. Periostracum pale yellow to darkly pigmented. Protoconch of 1.4-1.5 whorls; microsculpture of uniform, weak pits and faint, spirally arranged wrinkles. Teleoconch of 3.2-3.8 convex whorls, sculpture faint on some specimens, prosocline growth rings present; periphery of last whorl evenly rounded. Inner lip medium thickness and width, columellar swelling absent. Outer lip prosocline. Umbilicus small (< 0.1 mm wide).

Table 5.9. Shell dimensions of morphotype C.

Shell dimensions	AW	SW	SL	WB	BW	AL
	0.92	1.63	2.44	1.28	1.79	1.16
	0.94	1.64	2.46	1.30	1.78	1.18
	0.95	1.64	2.48	1.30	1.79	1.21
	0.90	1.58	2.39	1.25	1.76	1.14
	0.91	1.59	2.41	1.26	1.76	1.15
	0.94	1.63	2.45	1.29	1.78	1.17
Mean	**0.93**	**1.62**	**2.44**	**1.28**	**1.78**	**1.17**

* Note: shell measurement in table are for unsexed individuals

Head-foot. Pigmentation on whorls posterior to body whorl and on pallial roof between cephalic tentacles, faint medial longitudinal streak on cephalic tentacles, with some pigmentation on single band between cephalic tentacles.

Non-genital anatomy. Pallial tentacle absent. Ctenidium rudimentary, in about anterior 2/3 of pallial cavity, 10-16 filaments, (some very faint), most specimens with 10-12 filaments, broader than high with apices to the right. Osphradium elongate, narrow, anterior end simple. Hypobranchial gland, well developed, ridged. Rectum weakly to strongly arched, with faecal pellets oblique to variably arranged. Anus near pallial edge. Renal organ not extending forward into pallial roof, renal gland circular. Stomach with small caecum.

Male reproductive system. (Fig 5.7*K*, 5.8*H*). Testis of 1.3-1.4 whorls. Seminal vesicle beneath less than anterior1/3 of first whorl of testis behind stomach; not conspicuously coiled over stomach. Prostate gland occupying 1/2 to 2/3 of pallial roof, banana-shaped, with thick ventral wall, closed; vas deferens opening anteriorly. Pallial vas deferens straight. Penis medium size, with distal end medium length, tapering, simple, without papilla; medial section parallel sided, of medium length, penial duct straight to weakly undulating in medial and basal sections; base moderately wide, with moderate to strong folds.

Female reproductive system. (Fig 5.6*F*). Ovary simple, occupying about 0.4-0.5 whorls. Proximal coiled oviduct with single bend, with initial U bend orientated obliquely backwards; bound in connective tissue, of soft, glandular appearance, not extending to posterior edge of bursa copulatrix; straight distal to seminal receptacle. Coiled oviduct and bursal duct joining in front of posterior pallial wall at posterior pallial wall. Bursa copulatrix moderate, not extending to posterior wall; globular. Bursal duct arising from ventral edge of the bursa. Seminal receptacle pyriform, between posterior and ventral edge of bursa copulatrix on most specimens (Fig 5.6*H*). Half to 2/3 of albumen gland in front of posterior wall. Capsule gland intermediate thickness, about 2/3 length of albumen gland. Ventral channel with anterior vestibule. Genital opening anterior to capsule gland, small.

Remarks

This morphotype occupies only a few streams in the Castra Rivulet catchment and occurs infrequently. It differs from morphotypes B and D in the Castra group by possessing a single bend in the coiled oviduct, a rudimentary ctenidium with few filaments and a well developed, ridged, hypobranchial gland.

Morphotype D
(Figure 5.4*D,* 5.7*N*-*O*, 5.8*I*)

Material Examined

From Ca7, first order stream, tributary of Deep Gully Creek, on Ghost Hole Rd, Upper Castra, northern Tas., 427352 5422663 GDA. *Additional material*. Ca10, second order stream, tributary of Castra Rivulet, off Flints Rd, Nietta, northern Tas., 424592 5422263.

Diagnosis

Differs from morphotypes B and C of the Castra group in lack of protoconch pitting; closed umbilicus, number of ctenidium filaments intermediate between sp B and C; prostate shape, and long distal end on penis. *Only males recorded.*

Description

Shell. (Fig 5.4*D*). Conical, 2.56-2.64 mm in length; 1.66-1.69 in width, SW/SL of 0.64-0.65 and AL/SL of 0.47. Periostracum thin, colourless to pale yellow. Protoconch of 1.3-1.4 whorls; microsculpture uniform, of weak pitting. Teleoconch of 3.5 to 3.8 convex whorls, sculpture faint, prosocline growth rings present on some specimens; periphery of last whorl evenly rounded. Inner lip medium thickness and width, columellar swelling absent. Outer lip prosocline. Umbilicus small to closed (<0.1 mm wide).

Table 5.10. Shell dimensions of morphotype D.

Shell dimensions	AW	SW	SL	WB	BW	AL
	1.00	1.69	2.56	1.41	1.91	1.23
	1.00	1.66	2.64	1.40	1.91	1.21
	1.01	1.68	2.60	1.40	1.91	1.22
	1.02	1.69	2.62	1.42	1.92	1.23
	1.01	1.67	2.61	1.40	1.90	1.22
	1.03	1.69	2.63	1.42	1.92	1.24
Mean	**1.01**	**1.68**	**2.61**	**1.41**	**1.91**	**1.23**

* Note: shell measurement in table are for unsexed individuals

Head-foot. Dark pigmentation on whorls posterior to body whorl and on pallial roof between cephalic tentacles, medial longitudinal streak on cephalic tentacles, strong band of pigment between cephalic tentacles.

Non-genital anatomy. Pallial tentacle absent. Ctenidium wide, extending between 2/3 to entire length of pallial cavity, 15-19 filaments, apices on right. Osphradium medium width, oval, anterior end simple. Hypobranchial gland moderately developed, surface smooth. Rectum with prominent S-shaped arch, with faecal pellets variably arranged, longitudinally to obliquely. Anus near pallial edge. Renal organ extending forward about 1/2 length of renal gland into pallial roof, renal gland orientation variable. Stomach with small caecum.

Male reproductive system. (Figs 5.7*N-O*, 5.8*I*). Testis of 1.3 whorls. Seminal vesicle beneath more than anterior 1/3 of first whorl of testis behind stomach; coiled over stomach and forming large coiled mass behind stomach. Prostate gland occupying 2/3 of pallial roof, elongate pyriform, broadly oval to circular in section, with thin ventral wall; vas deferens opening anteriorly. Pallial vas deferens straight. Penis moderate-sized, with distal end long, tapering, simple, without papilla; medial section of medium length, tapering, with non-glandular lobe on convex edge of medial section, with straight to weakly undulating penial duct in medial and basal sections; base moderately wide, with moderate to strong folds.

Remarks

No females of this morphotype were recorded from 15 dissections of snails collected from two streams. This morphotype is most similar in shell morphology to morphotype B, although SW/SL is marginally higher and the AL/SL ratio is at the lower end of the morph B range. Pigmentation on this morph is also darker and more widespread, occurring on the pallial roof, cephalic tentacles and between the cephalic tentacles. The hypobranchial gland is well developed and contains ridges, a character not recorded on the other Castra morphotypes. Like morphotype B, this morph also contains a penial lobe, however, it is uncoloured and appears to be non-glandular, but further work is required for confirmation of this character.

5.3.4 Remarks on Castra Rivulet group

Of the four morphotypes recorded, morph A was uniquely different, being the largest, possessing an ovate shell and was the only morphotype to lack pigmentation. Morphotype A shares some anatomical characters with *Beddomeia wilmotensis*, but differences include: fewer ctenidial filaments (12-16, compared with 16-18 for *B. wilmotensis*), different size and shape of ctenidial filaments as well as the length of the ctenidium in the pallial cavity. Morphotype A also shares some similar characters with *B. inflata*, for example the well developed hypobranchial gland and shape of the prostate. Therefore the relationship between the named species and morphotype A is unclear.

Pigmentation on morph B differs from morphotypes C and D by being limited to the dorsal surface of the upper two spire whorls, whereas the pallial roof and cephalic tentacles are pigmented in morph C and darkly pigmented in morph D. No females of morph D were recovered. While it is possible that morph B is sexually dimorphic and that morph D are a subset of males of morph B, anatomical differences do exist including penial characters and structure of the hypobranchial gland, therefore it is more likely that females of morph D exist.

Egg capsules recovered from Castra Rivulet sites were conspicuous on darker material such as basalt rocks and woody debris. *Beddomeia* occur sympatrically in each of the streams, therefore determination of the morph to which they belong is not possible. Capsules were spherical, dome-shaped to flat topped, with a broad attachment base and covered with fine sand grains or composed of shredded woody material when constructed on woody debris; they were 0.6-0.85 mm in maximum length and contained single eggs (Figure 5.5). The use of wood fragments as construction material for egg capsules has not previously been recognised in *Beddomeia*.

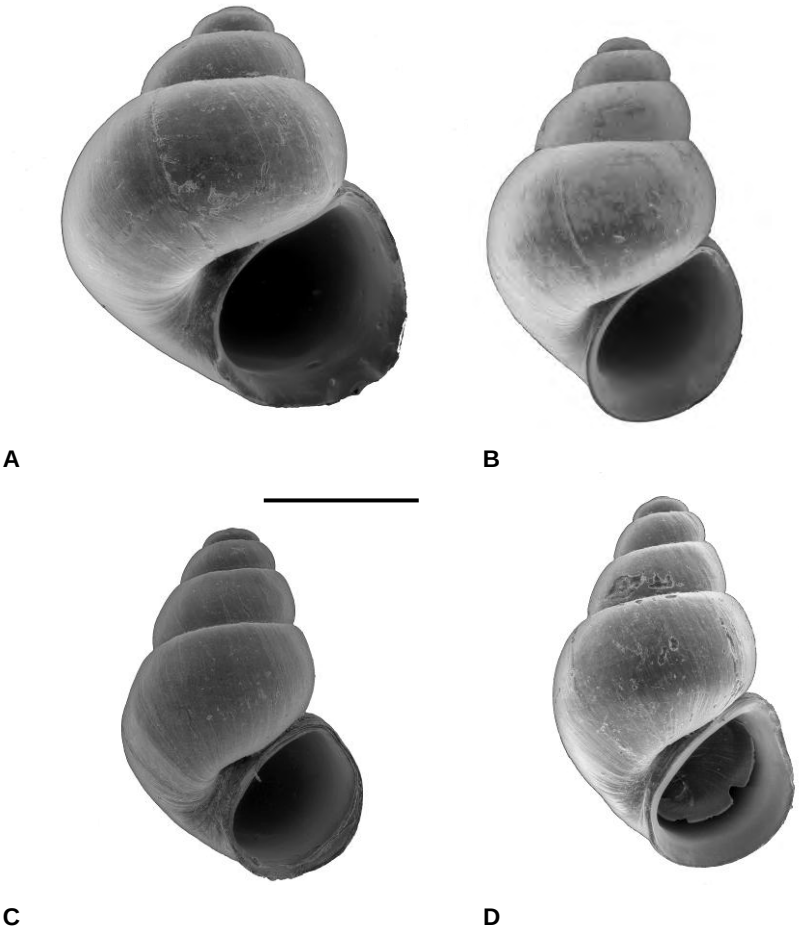


Figure 5.4. Shells of species of *Beddomeia* from Castra Rivulet: ***A***, morphotype sp A; ***B***, morphotype B; ***C***, morphotype C; ***D***, morphotype D. Scale bar: 1 mm.

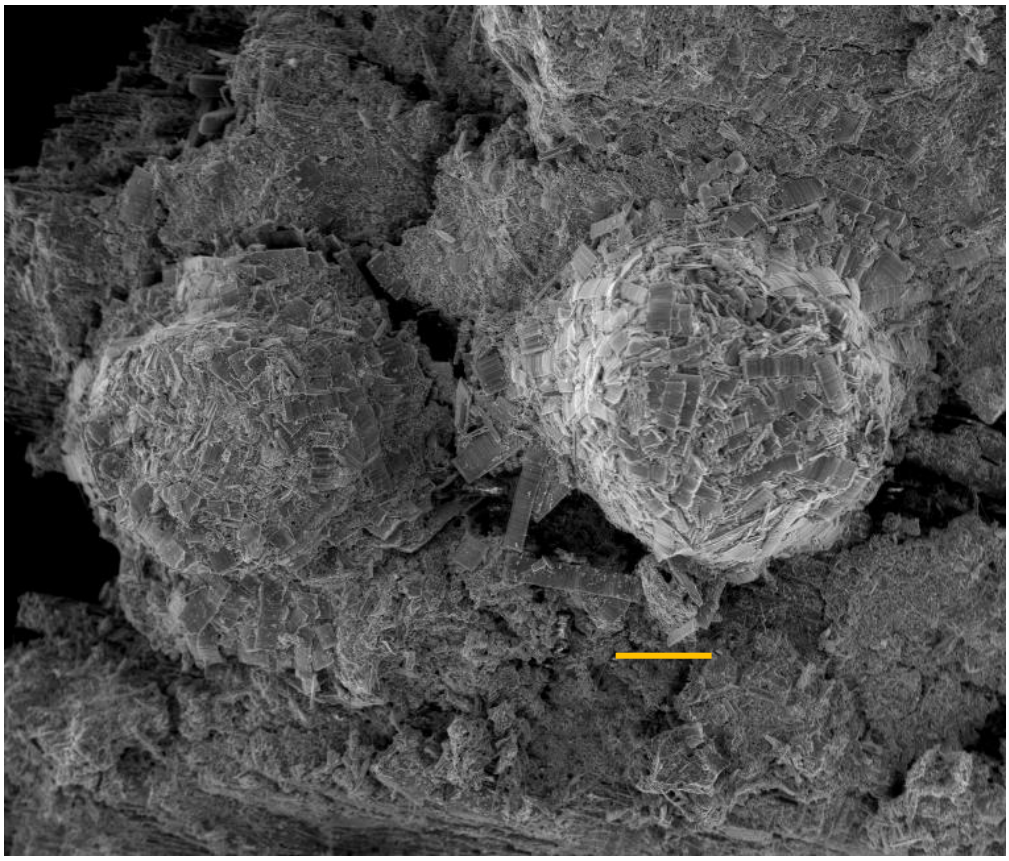


Figure 5.5. *Beddomeia* spp. egg capsules on woody debris, Castra catchment. Scale 300 µm.

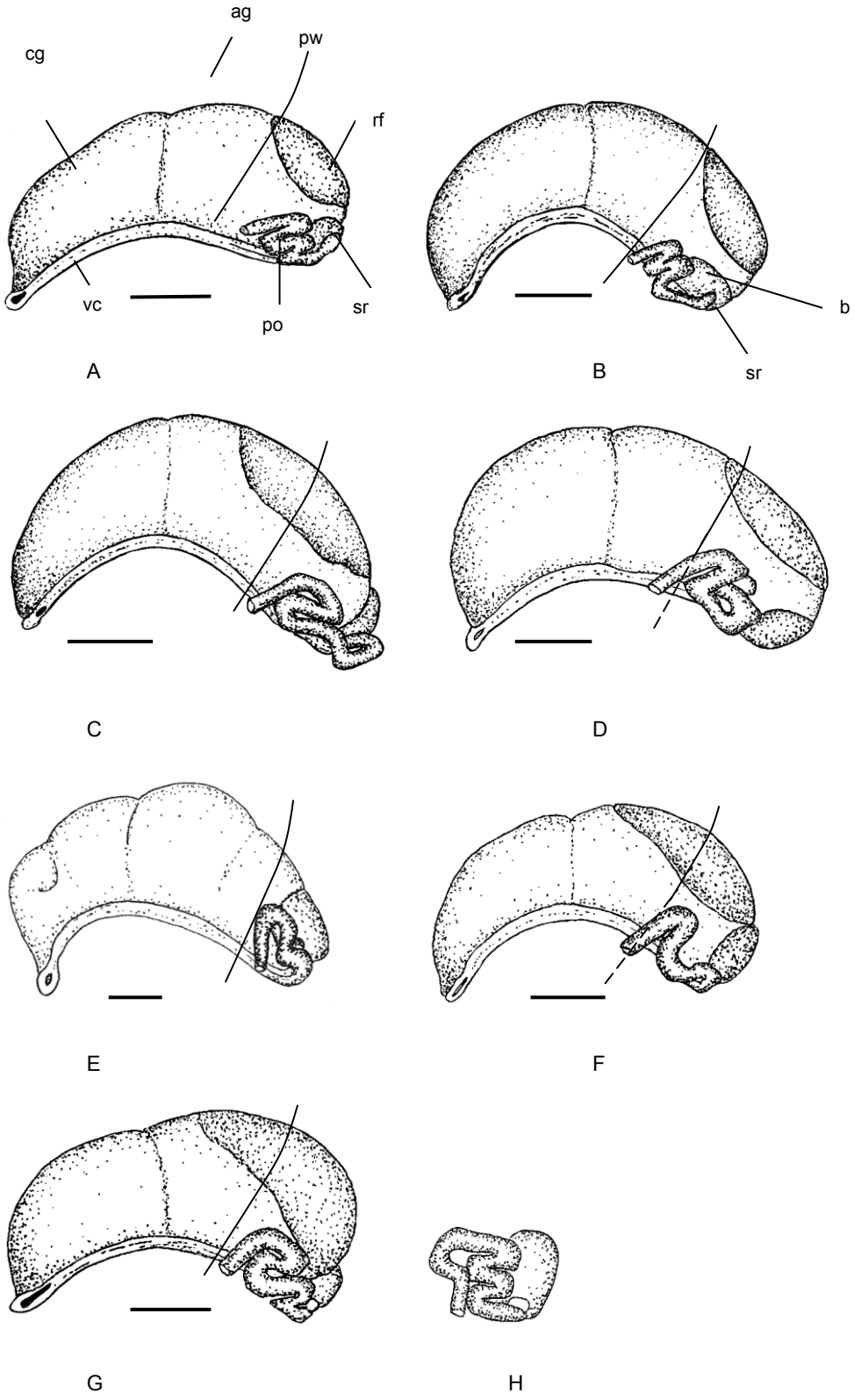


Figure 5.6. Female genitalia of *Beddomeia* morphotypes: ***A-B***, morph 1; ***C***, morph 2; ***D***, morph 6; ***E***, morph A; ***F*** morph C; ***G-H***, morph B [morph 3 and morph D (males only)]. ag, albumen gland, b, bursa copulatrix, cg, capsule gland, do, distal part of coiled oviduct, po, proximal coiled oviduct, pw, posterior pallial wall, rf, rectal furrow, sr, seminal receptacle. Scale bar: 250 µm.

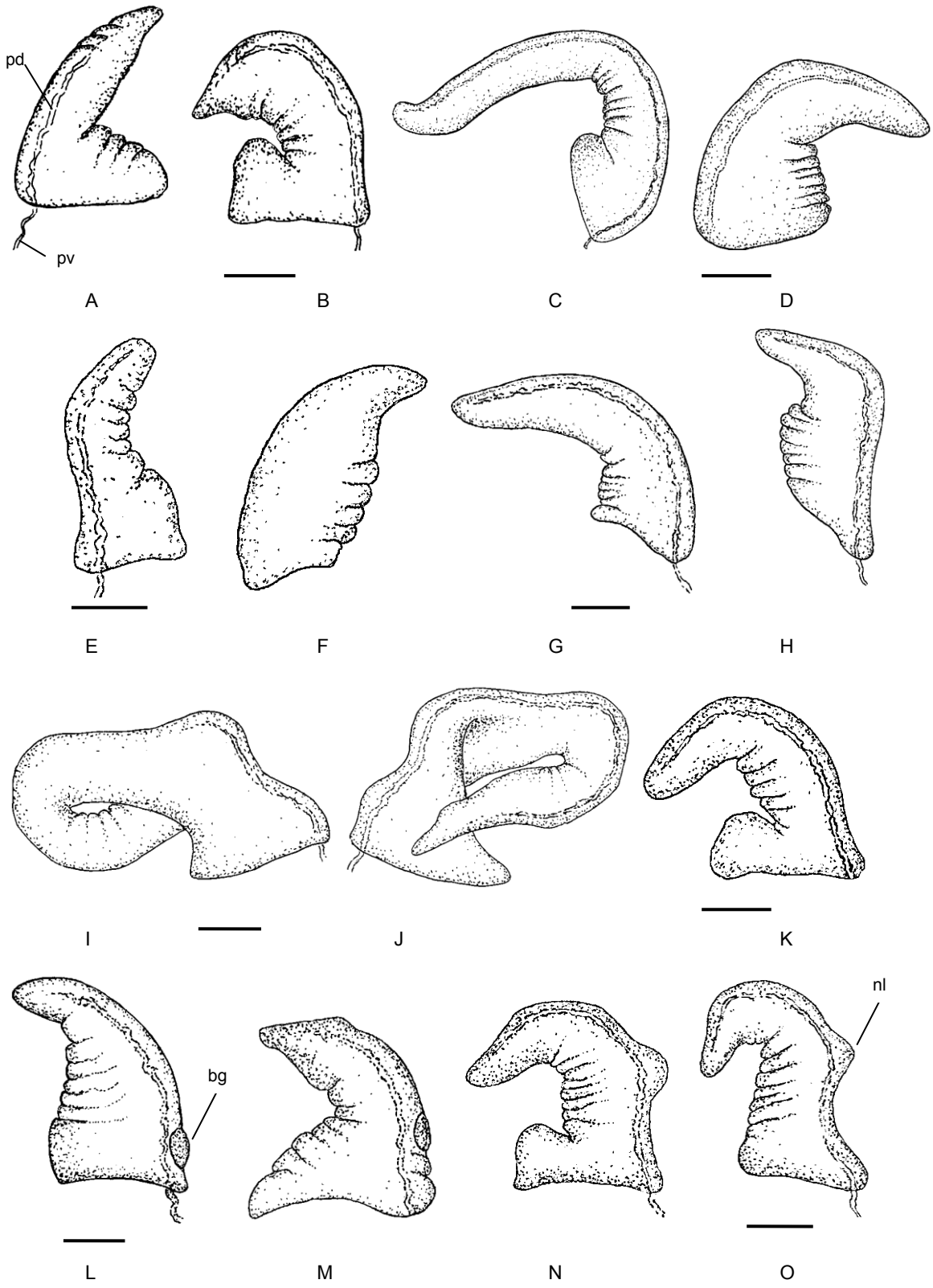


Figure 5.7. Male genitalia: Penes of *Beddomeia* morphotypes: ***A*-*B***, morph 1; ***C*-*D***, morph 2; ***E*-*F***, morph 3, ***G*-*H***, morph 6; ***I*-*J***, morph A; ***K***, morph C; ***L*-*M***, morph B and ***N*-*O***, morph D. bg, brown penial gland, nl, non-glandular lobe, pd, penial duct, pv, pallial vas deferens. Scale bar: 250 µm.

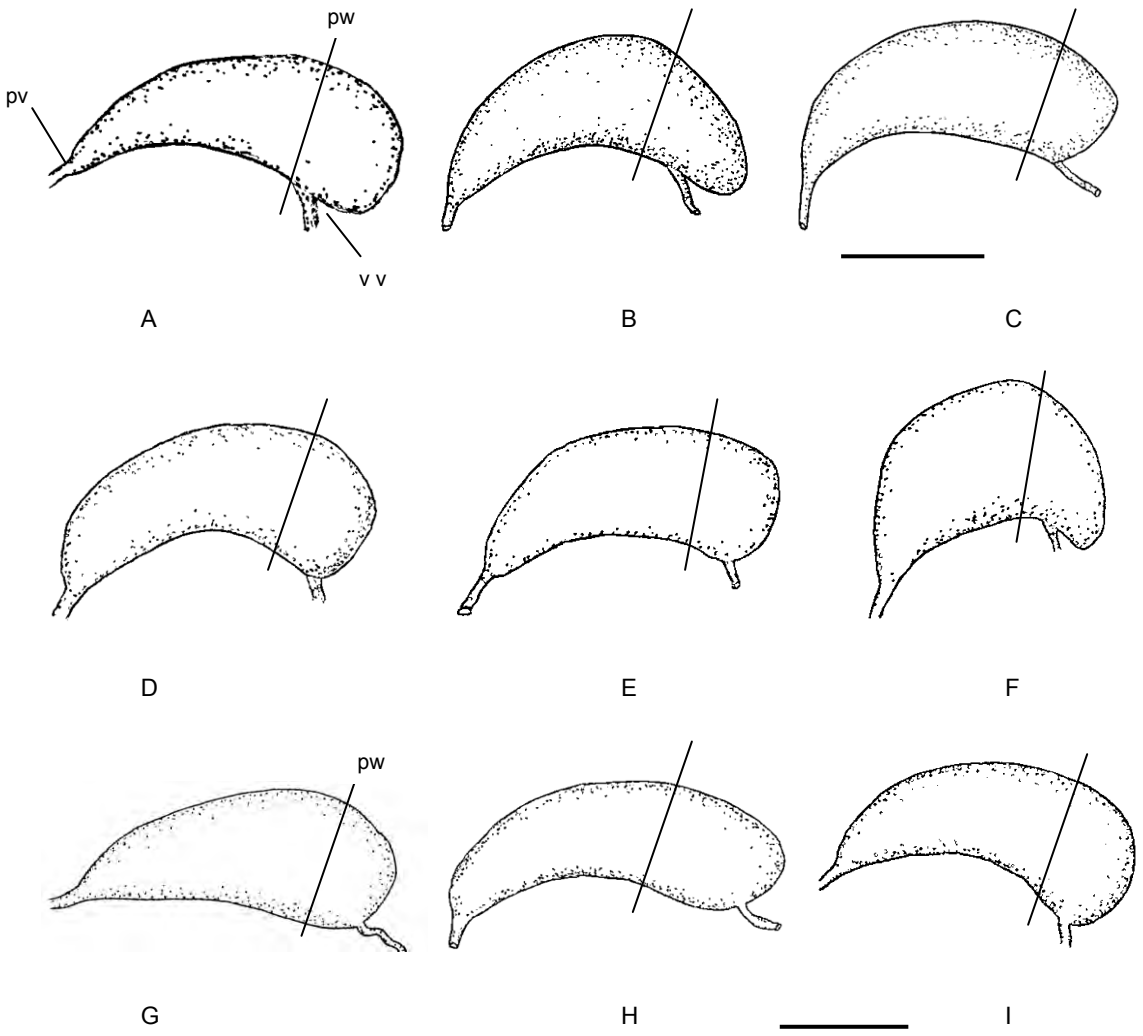


Figure 5.8. Prostate glands of *Beddomeia* morphotypes: ***A***, morph 1; ***B***-***C***, morph 2; ***D***, morph 3; ***E***-***F***, morph 6, ***G***, morph A; ***H***, morph C and ***I***, morph D (same for B). pv, pallial vas deferens, pw, pallial wall (diagonal line indicates the position of pallial wall, vv, visceral vas deferens. Scale bar: 500 µm.

Table 5.11. Comparative summary of the key morphological differences between each morphotype.

Morphotype	Shell dimensions											Head-foot	Ctenidium				Osphradium	
	AW	SW	SL	WB	BW	AL	SW/SL	AL/SL	Protoconch	Teleoconch	Umbilicus	Pigmentation	Filaments	Position	Width	Apices	Shape	Length
A	1.83	3.21	3.96	2.68	3.25	2.17	0.77-0.82	0.54-0.59	1.5	3.0-3.5	0-0.2	none	12 to 15	intermediate	broader than high	central	oval to elongate	small
B	1.07	1.74	2.78	1.55	2.03	1.33	0.62-0.63	0.47-0.49	1.5	3.5	0-0.1	dorsal surface of upper spiral whorls	18 to 22	length of PC	wide	right	oval to elongate	medium
C	0.93	1.62	2.44	1.28	1.78	1.17	0.66	0.48-0.49	1.4-1.5	3.2-3.8	<0.1	on upper spiral whorls except BW; on, and banding btw, CT.	10 to 16	rudimentary (anterior 2/3)	broader than high	right	narrow	medium
D	1.01	1.68	2.61	1.41	1.91	1.23	0.64-0.65	0.47	1.3-1.4	3.5-3.8	<0.1	dark pigmentation on upper spiral whorls except BW; on, and dark banding btw, CT	15 to 19	anterior 2/3 to full length	wide	right	oval	medium
1	1.02	1.9	1.94	1.44	1.68	1.09	0.94-1.01	0.53-0.59	1.4-1.5	2.7-3.0	0.3-0.35	on body including BW, CT, PR and btw CT	16 to 18	length of PC	narrow to medium	right	elongate, wider at anterior end	medium
2	1.04	1.85	1.69	1.36	1.6	1.14	1.08-1.10	0.66-0.67	1.5	2.2-2.6	0.3-0.35	on body including BW, CT, PR and btw CT	14 to 19	length of PC	narrow	right	elongate	medium
3	0.84	1.42	1.78	1.25	1.53	0.93	0.78-0.81	0.51-0.55	1.3-1.4	3.0-3.2	0-0.1	on body including BW, CT, PR and btw CT	15 to 19	length of PC	wide	right	elongate	medium
6	0.96	1.77	1.81	1.34	1.61	1.16	0.94-0.99	0.63-0.64	1.4-1.5	3.0-3.2	0.32-0.35	on body including BW, CT, PR and btw CT	11 to 18	length of PC	narrow	right	elongate, wider at anterior edge	medium

Morphotype	Hypobranchial gland		Rectum			Testis	Prostate		Penis				Ovary			
	Development	Surface	Shape	Pellet orientation	Anus	Whorls	Shape	Posterior vas deferens	Shape	Size	Folds	Penial duct	Whorls	Proximal coiled oviduct bends	Coiled oviduct - bursa duct junction	Capsule gland wall
A	Thick	smooth	long S-shape	oblique	near pallial edge	1	oval to elongate	straight	tapering	medium	weak to moderate	straight to undulating	0.4-0.5	3	at pallial wall	thin
B	well developed	ridged	prominent arch	oblique	near pallial edge	1.5	elongate pyriform	straight	tapering	medium	weak	weakly undulating	0.5	2	in front of pallial wall at posterior wall	intermediate
C	well developed	ridged	weak to strong arch	oblique to variable	near pallial edge	1.3-1.4	banana-shape	straight	tapering	medium	weak to moderate	straight to undulating	0.4-0.5	1	in front of pallial wall at posterior wall	intermediate
D	Moderate	smooth	prominent S-shaped arch	variable	near pallial edge	1.3	banana-shape to broadly ovate	straight	tapering	medium	weak	straight to undulating				
1	Moderate	smooth	moderate arch	oblique to longitudinal	intermediate	1.5-2.0	elongate pyriform	coiled	tapering	medium	moderate	straight to undulating	0.3-0.5	2	in front of pallial wall at posterior wall	swollen
2	Weak	smooth	slight to moderate arch	oblique	intermediate	1.5-1.8	pyriform to banana-shape	undulating	tapering	medium	moderate to strong	straight	0.5	2	at pallial wall	swollen
3	Weak	smooth	strongly arched (few double arched)	oblique to longitudinal	at pallial edge	1-1.4	pyriform	straight	broadly triangular	small to medium	moderate	weakly undulating				
6	weakly to moderate	smooth	slight to moderate arch	longitudinal	intermediate	1.5-1.9	pyriform, oval	straight	tapering	medium	weak to moderate	straight	0.4-0.5	3	in front of pallial wall at posterior wall	swollen

* Key: AW, aperture width, SW, shell width, SL, shell length, WB, body whorl, BW, body width, AL, aperture length, CT, cephalic tentacles, PC, pallial cavity and PR, pallial roof.

5.4 Discussion

The principle aim of this chapter was to determine whether sufficient anatomical differences exist between the morphotypes identified in previous chapters to support the number of morphotypes recognised from shell morphology (in chapter 3 and 4). A subset of anatomical characters, recognised as defining for *Beddomeia* species by Ponder *et al.* (1993), were used to examine potential differences between the morphotypes. Partial descriptions for each morphotype are provided to assist in distinguishing the morphotypes and to provide supporting evidence for the ecological study in Chapter 3. It was not intended to produce complete morphological descriptions of these morphs and morphometric analyses of shell characters were not conducted.

Initially, the identification of morphotypes in Chapter 3 was based on shell characteristics, and as expected from the findings of Ponder *et al.* (1993), there is a clear and distinct separation of species and morphotypes between the Castra Rivulet and Groom River catchments. Plasticity of intraspecies shell characters due to the convergent nature of the Hydrobiidae has been well documented (e.g. Ponder *et al.* 1993, Hershler and Ponder 1998), indicating that the shell features alone are insufficient for species-level determinations within this family. Examination of the anatomical characters of the morphotypes has revealed that the initial hypothesis of four morphotypes in each catchment was an overestimate and should be reduced to two species at Groom River and possibly three species in the Castra Rivulet catchment.

Groom River catchment morphotypes

It is likely that two species of *Beddomeia* exist within the Groom River catchment morphotypes, morphs 1, 2 and 6 being variants of the same „species' (*B. tasmanica*) while morph 3 displays some characteristics similar to *B. briansmithi* and at least one other *Beddomeia* species, although only males of morph 3 were recorded. Support for combining morphotypes 1, 2 and 6 into a single species is provided by the similarity of most anatomical traits between the three morphs, while the shell variability fits within the range of measurements present in the re-description of *B. tasmanica* in Ponder *et al.* (1993). Additionally, all three morphotypes occur at the Type locality from which the species was originally collected (Ponder *et al*. 1993, K. Richards unpublished data) and the diagrams of *B. tasmanica* presented in Ponder *et al.* (1993) closely resemble morphs 1 and 6.

Morphotype 3 is anatomically distinguishable from the other Groom River morphs, both in shell shape (mature specimens possessing a separation of the final body whorl and closed umbilicus)

and in the position and arching of the rectum. It also shares features in common with at least two described species: *B. briansmithi* and *B. fromensis* (shell characters), as well as displaying some similarity to *B.* cf. *minima*. However, this morphotype also displays differences to each of the named species that fall outside the variation in their descriptions, and therefore it cannot be assigned to any currently named species. While a few species of other genera of Hydrobiidae, (for example *Austropyrgus* (Clark *et al.* 2003)) possess a separation of the final body whorl, morph 3 is the first *Beddomeia* to display this trait. Together, the anatomical differences observed indicate that morph 3 is a candidate for species status.

Only male specimens were recorded for this morph. However, adult specimens were rare in samples obtained from Groom River streams (Chapter 3). Although it is possible that this morph is protandric, it would be the only example of this in *Beddomeia*, and as the dissected specimens were all mature adults (and male), protandry is unlikely, males occurring before females, but can't be totally discounted. Further surveys and dissections are required to investigate this issue; but it is likely that females will be detected with the recovery of more specimens.

Castra Rivulet catchment morphotypes

External shell features such as inner lip thickness, protoconch and teleoconch sculpturing, the size, shape and number of teleoconch whorls, as well as internal characters, suggest that the Castra morphotypes represent at least three species; morphotype A (*B.* cf *wilmotensis*), a species containing the group of morphotypes B and D (closely related to *B. hallae* and *B. fallax* based on the description given in Ponder *et al.* (1993)) and a species comprised of morphotype C, although the relationship between morphotypes B and D requires further investigation.

Morphotype A is the most distinctive of the four morphs identified in the Castra Rivulet catchment. Its size, shape and anatomy clearly distinguish it as a separate species from the other Castra morphs; however, morph A possesses a set of anatomical characters which do not completely match any of the currently described species in Ponder *et al.* (1993). Rather, morph A displays a set of traits that represent a combination of two species, *B. wilmotensis* and *B. inflata*. Current available information (Ponder *et al.* 1993), indicates that these two species occupy separate, but geographically close, catchments. However, the work conducted by Ponder *et al.* (1993) was not comprehensive, and the intricacies of small scale geographical interactions were not detectable due to the scale of sampling. It is therefore possible that morph A is the intermediate between *B. wilmotensis* and *B. inflata*, although it is more likely that it is more similar to the ancestral form that gave rise to both species, a supposition supported by its distribution throughout the Castra Rivulet catchment (six widely separated tributaries)

compared with the known distributions of *B. wilmotensis* and *B. inflata* which occur nearby, but fall outside this area. Further work is required to unravel this relationship.

Limited, but possibly significant variation in morphological traits do exist between morphotypes B and D; but, given that one of these is represented by individuals of only a single sex, it is possible that morph B is sexually dimorphic and that morph D represent males of morph B. This is unlikely, however, as some males of morphotype B have been identified and shown to possess shell characters similar to the females of that morph. The anatomical differences are, however, probably sufficient to warrant retaining morphs B and D as separate species. Insufficient information is currently available to elucidate the relationship between these morphs, and further work is required to determine whether females of morphotype B exist. Due to the limited number of anatomical character differences recognised between morphotypes B and D, more complete dissections, which include anatomical characters not used in this study, are also required to fully explore this relationship. The DNA investigations conducted as part of this thesis (Chapter 6) will further assist in understanding this relationship.

Morphotype C possesses a series of distinguishing anatomical characters from those of morphs B and D, the most obvious being an extended aperture on the shell, but other features include a single bend in the coiled oviduct, a rudimentary ctenidium with few filaments and a well developed, ridged, hypobranchial gland in morph C. The anatomical characters of morph C do not correspond to any described species from the Castra region, or beyond, and this morphotype occurs in low abundance in only seven of the 18 Castra Rivulet streams. It is therefore likely that morphotype C is a separate species from the other Castra Rivulet morphotypes and it is expected that the DNA investigation in Chapter 6 will confirm this finding.

5.5 Conclusion

The descriptions presented here form only part of the more detailed morphological descriptions required for publication, but are sufficient to realize the intent of this chapter, which was to determine whether sufficient anatomical differences exist between the morphotypes to validate their existence.

The findings indicate that insufficient morphological differences exist to support the number of morphotypes originally identified in Chapter 3, although two *Beddomeia* species from Groom

River and three species from the Castra Rivulet catchment are recognisable using the anatomical characters investigated in this study.

Two morphotypes were represented by specimens of a single sex (morphotypes D and 3), and while it is possible that they may be protandric, it is likely that further sampling and subsequent dissections will recover the missing forms.

5.6 Appendix

Appendix A. Taxonomic history of Beddomeia Petterd

Genus *Beddomeia* Petterd

Beddomeia Petterd, 1889: 73. Type species by subsequent designation (Cotton 1943a: 124). *Tasmaniella launcestonensis* (Johnston, 1879) = *Amnicola launcestonensis*.
Brazieria Petterd, 1889: 76. Type species *Ampullaria tasmanica* Tenison-Woods, 1877.
Petterdiana Brazier, 1896: 105, replacement name for *Brazieria* Petterd, 1889, non *Brazieria* Ancey, 1887.
Tasmaniella Ancey, 1898: 148, new name for *Beddomeia* Petterd, 1889, non *Beddomea* Nevill, 1878 (unnecessary replacement name).
Pseudoampullaria Ancey, 1898: 148, replacement name for *Brazieria* Petterd, 1889, non *Brazieria* Ancey, 1887.
Petterdiella Pilsbry, 1900: 144 (error for *Petterdiana*).
Beddomena Iredale, 1943: 202. Type species *Beddomeia bellii* Petterd, 1889.
Valvatasma Iredale, 1943: 203. Type species *Valvata tasmanica* Tenison-Woods, 1876.

Current taxonomy of *Beddomeia*

***single-indented taxa are synonyms**
****double-indented taxa are subspecies**
*****double-indented taxa in brackets no longer recognised**

Beddomeia acheronensis Ponder & Clark, 1993
    Beddomeia acheronensis absona Ponder & Clark, 1993
    Beddomeia acheronensis acheronensis Ponder & Clark, 1993
Beddomeia angulata Ponder & Clark, 1993
Beddomeia averni Ponder & Clark, 1993
Beddomeia bellii Petterd, 1889
Beddomeia bowryensis Ponder & Clark, 1993
Beddomeia briansmithi Ponder & Clark, 1993
Beddomeia camensis Ponder & Clark, 1993
Beddomeia capensis Ponder & Clark, 1993
Beddomeia fallax Ponder & Clark, 1993
Beddomeia forthensis Ponder & Clark, 1993
Beddomeia franklandensis Ponder & Clark, 1993
Beddomeia franklinensis Ponder & Clark, 1993
Beddomeia fromensis Ponder & Clark, 1993
Beddomeia fultoni Ponder & Clark, 1993
Beddomeia gibba Ponder & Clark, 1993
Beddomeia hallae Ponder & Clark, 1993
Beddomeia hermansi Ponder & Clark, 1993
Beddomeia hullii Petterd, 1889
Beddomeia inflata Ponder & Clark, 1993

Beddomeia kershawi Ponder & Clark, 1993
Beddomeia kessneri Ponder & Clark, 1993
Beddomeia krybetes Ponder & Clark, 1993
Beddomeia launcestonensis Johnston, 1879
    Amnicola launcestonensis Johnston, 1879
        (*Beddomeia launcestonensis minima* Petterd, 1889)
        (*Beddomeia launcestonensis tumida* Petterd, 1889)
Beddomeia lodderae Petterd, 1889
Beddomeia mesibovi Ponder & Clark, 1993
Beddomeia minima Petterd, 1889
        (*Beddomeia launcestonensis minima* Petterd, 1889)
Beddomeia pallida Ponder & Clark, 1993
Beddomeia paludinella Reeve, 1857
    Littorina paludinella Reeve, 1857
        Beddomeia paludinella paludinella (Reeve, 1857)
        Beddomeia paludinella levenensis Ponder & Clark, 1993
Beddomeia petterdi Ponder & Clark, 1993
Beddomeia phasianella Ponder & Clark, 1993
Beddomeia protuberata Ponder & Clark, 1993
Beddomeia ronaldi Ponder & Clark, 1993
Beddomeia salmonis Ponder & Clark, 1993
Beddomeia tasmanica Tenison-Woods, 1876
    Valvata tasmanica Tenison-Woods, 1876: 82.
    Valvatasma tasmanica Iredale, 1943: 203.
    Beddomeia tasmanica Smith, 1992: 45.
Beddomeia topsiae Ponder & Clark, 1993
Beddomeia trochiformis Ponder & Clark, 1993
Beddomeia tumida Petterd, 1889
        (*Beddomeia launcestonensis tumida* Petterd, 1889)
Beddomeia turnerae Ponder & Clark, 1993
Beddomeia waterhouseae Ponder & Clark, 1993
Beddomeia wilmotensis Ponder & Clark, 1993
Beddomeia wiseae Ponder & Clark, 1993
Beddomeia zeehanensis Ponder & Clark, 1993

Chapter 6

Molecular phylogenetic examination of *Beddomeia* species (Hydrobiidae: Mollusca)

Chapter 6 explores the phylogenetic relationships amongst members of the *Beddomeia* genus in an attempt to determine the systematics of a subset of the genus and thus test the current, morphologically-based taxonomy of *Beddomeia*. It also investigates the morphotypes described in Chapter 5 and discusses the speciation with respect to the molecular phylogenies.

6 Molecular phylogeny of and biogeography in *Beddomeia* species (Hydrobiidae: Mollusca) from Tasmania, Australia using CO1 and 16S mtDNA

6.1 Abstract

The caenogastropod family Hydrobiidae, now under review, is the most diverse of all freshwater gastropod families containing almost 400 valid generic names. While in Australia the family is not particularly well represented, morphological systematic studies have reported sufficient anatomical variation between snails from adjacent catchments or tributaries of catchments to indicate very local speciation within certain genera; a pattern particularly evident in the genera *Austropyrgus* and *Beddomeia* from south-eastern Australia. Genetic studies conducted on Hydrobiidae both internationally and on mainland Australia generally support previous morphological taxonomies, or else provide evidence for further subdivision of taxa; however, to date limited molecular phylogenetic studies of Tasmania's hydrobiid fauna have been undertaken.

In this paper we investigate the molecular phylogenetic relationships within an endemic radiation of hydrobiids (*Beddomeia*) from Tasmania in an attempt to address the question of validity of the alpha taxonomy of this genus. We review the findings in terms of the morphological taxonomy and geographical distribution of the species. Patterns of close species relationships are revealed by the presence of five well-supported clades; however the monophyly of *Beddomeia* is questioned by relationships with *Phrantela* and *Austropyrgus* in some of the analyses.

6.2 Introduction

The caenogastropod family Hydrobiidae is the most diverse of all freshwater gastropod families, containing almost 400 valid generic names and over 1,000 described species; thought likely to be an underestimate and could be as many as 4,000 species (Kabat and Hershler 1993, Strong *et al.* 2008). The Australian fauna is modest relative to the total number of genera, represented by only 18 genera, although most all but one are endemic, and the island of Tasmania supports eight genera; two of which, *Tatea* and *Ascorhis*, are predominantly estuarine

and coastal, with the remaining six genera occurring in freshwater. Four of these genera (*Beddomeia*, *Phrantela*, *Nanocochlea* and *Pseudotricula*) are endemic to Tasmania, while one, the genus *Austropyrgus,* contains a radiation of 76 species occurring across eastern Australia, almost half of which (31) are endemic to Tasmania (Ponder *et al.* 1989, Ponder *et al.* 1993, Clark *et al.* 2003, Perez *et al.* 2005). The remaining genus present is represented by a single species, *Potamopyrgus antipodarum*, introduced from New Zealand in the late 1800s (Ponder 1988) and now widespread, with a pattern of distribution closely resembling European colonisation.

The limited dispersal capabilities of the native species, resulting from a combination of their small size restricting mobility, food and habitat requirements, and reproductive methods provide opportunities for populations to become isolated (Ponder *et al.* 1994, Ponder 1997b, Ponder and Colgan 2002, Ponder and Walker 2003, Strong *et al.* 2008). Such isolation has been shown to result in small-scale allopatric speciation of several hydrobiid genera, for example *Austropyrgus* (formerly *Fluvidona*) in southeast Australia (Ponder *et al.* 1994, Clark *et al.* 2003) and *Fonscochlea* and *Jardinella* in the Great Artesian Basin (GAB) (Ponder *et al.* 1989, Ponder and Clark 1990, Ponder *et al.* 1995). Morphological systematics studies have shown that sufficient anatomical variation exists between *Beddomeia* from adjacent catchments to warrant application of the term „radiation' to describe rapid speciation events; this pattern is also reflected in the genus *Austropyrgus* (Ponder *et al.* 1993, Clark *et al.* 2003) across southeastern Australia, in *Fonscochlea* in the GAB (Ponder *et al.* 1989), and others, for example the subgenus *Pyrgulopsis* (*Natricola*) in North America (Hershler and Liu 2004) and *Tylomelania* (Gastropoda: Pachychilidae) in Indonesia (Rintelen *et al.* 2007).

Beddomeia species are not evenly dispersed across the landscape, with many species known only from single localities or individual tributaries of larger catchments, and some areas revealing high levels of speciation over relatively short distances. While the total number of described species in any given area is usually low, one or two species, some areas contain as many as nine species and subspecies, such as in the Castra region in central north (Figure 6.1). A review of the hydrobiids (*Beddomeia* and *Phrantela* species) listed on the Tasmanian *Threatened Species Protection Act*, *1995*, undertaken in 2008 and based principally on additional distributional information (Davies and Cook 2002; K. Richards, unpublished data) reduced the number of listed species to 41 (from 42). However, of the 41 species remaining listed, 14 were upgraded to either vulnerable or endangered. Owing to the large proportion of hydrobiid species listed under the Tasmanian *Threatened Species Protection Act*, *1995*, the difficulty of their identification (requiring specialist skills) and the serious consequences of

these listings for land managers, it is perhaps understandable that questions have been raised regarding the validity of the current taxonomy.

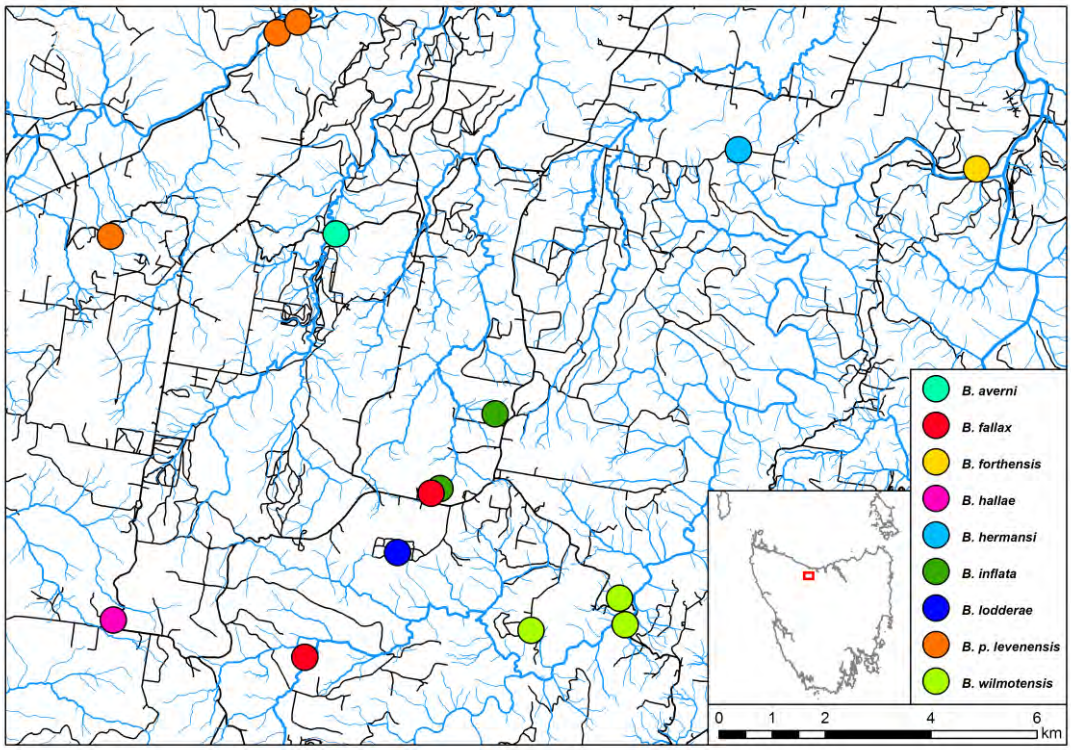


Figure 6.1. Example of the *Beddomeia* radiation near Castra, showing distribution of nine *Beddomeia* species in one 12 km by 20 km area of Tasmania. Coloured circles indicate approximate locations of species.

Molecular and allozyme investigations conducted on various freshwater mollusc groups to date have generally either supported phylogenies derived from previous morphological data (e.g. GAB studies which recognize the accepted taxonomy, with some synonymies as discussed in Perez *et al.* (2005), based on Ponder *et al.* (1996)), or else, have provided evidence for further taxonomic review, for example within the bivalve family Sphaeriidae, where Lee & Foighil (2003) determined the presence of five major monophyletic lineages in the cosmopolitan subfamily Sphaeriinae (*Pisidium* in North America, *Afropisidium* in South America, *Odhneripisidium* in Asia while *Sphaerium* and *Cyclocalyyx* are widely distributed genera occurring across North and South America, Europe, eastern Asia and Australia), instead of the three accepted through morphological studies.

The aim of this Chapter is to investigate the molecular systematics and biogeography of species of *Beddomeia* in an attempt to address the question of validity of the current morphological

taxonomy. To assist in this aim, partial segments of the cytochrome oxidase subunit 1 (CO1) and 16S gene regions for 19 ‚named' *Beddomeia* species and subspecies, collected from their type localities, one undescribed *Beddomeia* sp. (sp 11), one *Austropyrgus* species and two *Phrantela* species were sequenced. Nine *Beddomeia* morphotypes collected from the study catchments in Chapter 3 and adjacent catchments in northeastern Tasmania were also sequenced to address the question of identification of *Beddomeia* species based solely on shell morphology, given the recognized convergence of shell shape within the genus and between some species (Ponder *et al.* 1993). Previous sequences from *Beddomeia* species as well as those from several other Northern Hemisphere hydrobiid genera (*Cincinnatia*, *Hydrobia* and *Mercuria*) were obtained from Genbank (Bilofsky and Bunks 1988) to assist in determining relationships between presumably close and more distantly related genera and to assess the appropriateness of using *Pseudotricula eberhardi* and *Potamopyrgus antipodarum* as outgroups. Although Perez *et al.* (2005) sequenced 16S and CO1 gene regions for a handful of Tasmanian species (including *Beddomeia* (3), *Phrantela* (2), *Nanocochlea* (1) and *Pseudotricula* (1)) for inclusion in a study of the molecular phylogeny of spring-associated hydrobiids in the GAB, the study described here remains the first molecular phylogenetic investigation to have been specifically conducted on any Tasmanian aquatic hydrobiid genera.

6.3 Materials and methods

6.3.1 Specimens and vouchers

Hydrobiids examined in this study were collected from their type localities between September and December 2003, and additional sequences for a further 11 species were obtained from Genbank using BLAST (for details see http://www.ncbi.nlm.nih.gov) (Bilofsky and Bunks 1988, Altschul *et al.* 1990). The taxa analysed and their collection locations are presented in Table 6.1, locations are provided in GDA (for details see http://www.ga.gov.au/geodesy/datums/gda.jsp). Species locations for both collected and Genbank sequences are presented in Figures 6.2*a-d*. Individual sequences and the sequence alignment will be submitted to Genbank prior to the publication of this study. Morphotypes were allocated either letters or numerals (labelled *B.* sp. 1-5 and *B.* sp. A–D in figures presented in this chapter to reduce complexity of text on maps), signifying collection locations in the northeast and central north respectively.

Sequences for two ingroup species were obtained from GenBank for inclusion in the analyses: *Beddomeia hullii* CO1: AY622470, 16S: AY622404 and *Beddomeia minima* CO1: AY622458, 16S: AY622388. The additional sequences: *Nanocochlea* sp. 16S: AY622401, *Phrantela daveyensis tristis* CO1: AY622456, 16S: AY622386, *Phrantela marginata* CO1: AY622486, *Potamopyrgus antipodarum* CO1: AY631102, 16S: AY314009, *Pseudotricula eberhardi* CO1: AY622472, 16S: AY622403 and *Austropyrgus simsonianus* CO1: AY622468, 16S: AY622397 were also obtained for the analyses. Additional outgroups sequences obtained from Genbank were *Cincinnatia winkleyi* CO1: AF118370, 16S: AY622433, *Mercuria similis* CO1: AF367646, 16S: AF478393 and *Hydrobia acuta acuta* CO1: AF278809, 16S: AF478395. The GenBank sequence for *Beddomeia launcestonensis* CO1: AY622457, 16S: AY622387 was obtained but excluded from the final analyses due to dissimilarity with a second *B. launcestonensis* sequenced in this study, showing a closer relationship to *Phrantela* spp.

16S and CO1 datasets

A total of 42 sequences from hydrobiid species (including unnamed morphotypes) were sequenced or obtained from Genbank for the 16S gene (Table 6.2). CO1 sequences were obtained for 47 individuals (35 species, subspecies or morphotypes - including duplicates of species and Genbank sequences) were sequenced or obtained from Genbank for the CO1 gene (Table 6.2). Outgroups included in the analyses were *Potamopyrgus antipodarum*, *Pseudotricula eberhardi*, *Nanocochlea* sp., *H. acuta, C. winkleyi* and *M. similis*.

Combined dataset

The „combined' dataset included only hydrobiid taxa for which both CO1 and 16S gene sequences were obtained from the same individual, and included 33 „species' and morphotypes, 26 of which were named *Beddomeia* species, subspecies or morphotypes and one *Austropyrgus* (*A.* cf *lochi*), with the remaining sequences obtained from Genbank (Table 6.2). Outgroups *A.* cf *lochi*, *H. acuta, C. winkleyi* and *M. similis* were used in the analyses.

DNA sequences

Partial sequences of 16S rDNA and CO1 were obtained through polymerase chain reaction (PCR) using the 16S rDNA: **16SL** and **16SH** (Palumbi and Metz 1991) and CO1 primers: **CO1L1490F** and **CO1R2198R** (Folmer *et al.* 1994).

6.3.2 DNA extraction, PCR amplification, and sequencing

All samples used in this analysis were preserved in 80% ethanol. DNA was extracted from entire snails using a CTAB lysis buffer (Saghai-Maroof *et al.* 1984) and 5µL Proteinase K and

purified by phenol-chloroform extraction applying a standard protocol (Hillis and Mortiz 1990). Partial sequences of the CO1 and 16S rDNA genes were amplified using the following 50µL PCR reaction mix; 10 x buffer (670 mM Tris-HCl, pH 8.8, 166 mM $(NH_4)^2SO_4$, 4.5 % Triton X-100, 2 mg/ml gelatin), 1.5 mM MgCl, 200 µM dNTP's, 0.2 µM each Primer, 5 µg BSA, 1 unit Taq, distilled H_2O and 10-50ng DNA. The PCR thermal cycling conditions were: 95˚C for 3 min, 55.0˚C for 0.45 min (16S) or 52˚C for 0.45 min (CO1), 72˚C for 1 min, for 35 cycles followed by, 72˚C for 5 min, and a low temperature hold.

Several specimens were considerably smaller than the rest and did not provide sufficient yield of DNA for PCR, giving a weak amplification signal. Fluorometry was used to estimate PCR product concentrations of specimens on agarose gels. For samples displaying weak signals, the PCR protocol was modified to allow the addition of a larger volume of dilute DNA. Weak PCR product samples were re-extracted or discarded from the analysis. Morphotype *Beddomeia* sp. 5 (from St Columba Falls), *Beddomeia fromensis*, *Beddomeia ronaldi* and *Phrantela pupiformis* samples contained additionally replicated DNA material.

The DNA was purified using UltraClean™ PCR Clean-up™Kit (MoBio Laboratories Inc, http://www.mobio.com) and gene regions sequenced using the Beckman Coulter CEQ Dye Terminator Cycle Sequencing Kit according to the manufacturers specifications (Beckman Coulter Inc, http://www.beckmancoulter.com). Ethanol precipitation of samples was followed by resuspension in 28 µL Sample Loading Solution before samples were loaded onto a 96 titre plate for sequencing. Forward primer sequences used during sequencing reactions produced more reliable results on a preliminary subset of samples and was selected as the sequencing primer for the remainder of analyses.

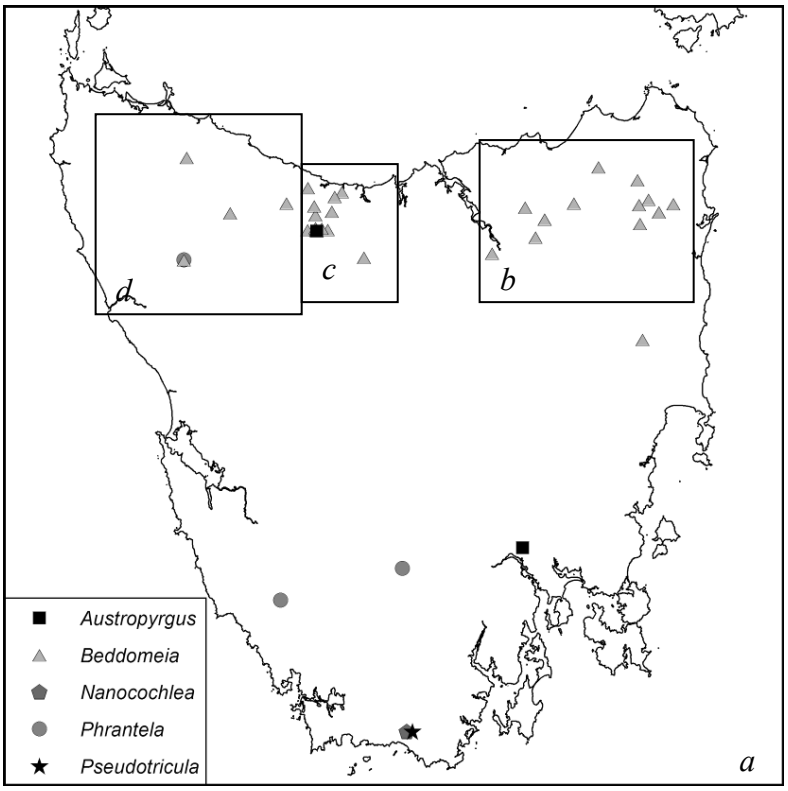


Figure 6.2*a*. Location of specimens obtained for this study.

Figure 6.2*b*. Location of northeast *Beddomeia* specimens obtained for this study. *B. sp. 1 – 5* are morphotypes recognised in Chapter 3. Scale 50 km.

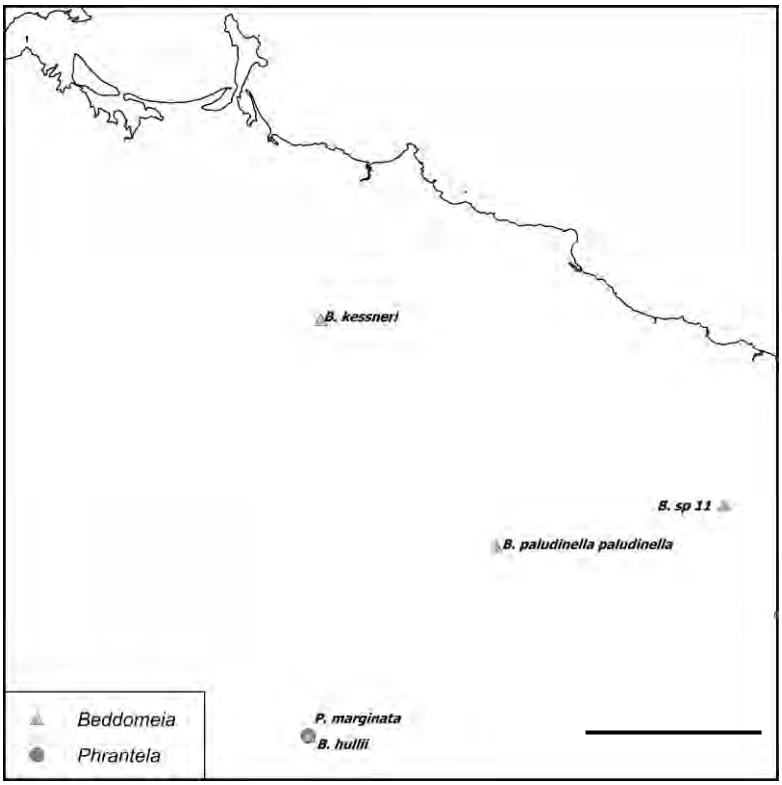


Figure 6.2*c*. Location of specimens obtained in northwest Tasmania. Scale 50 km.

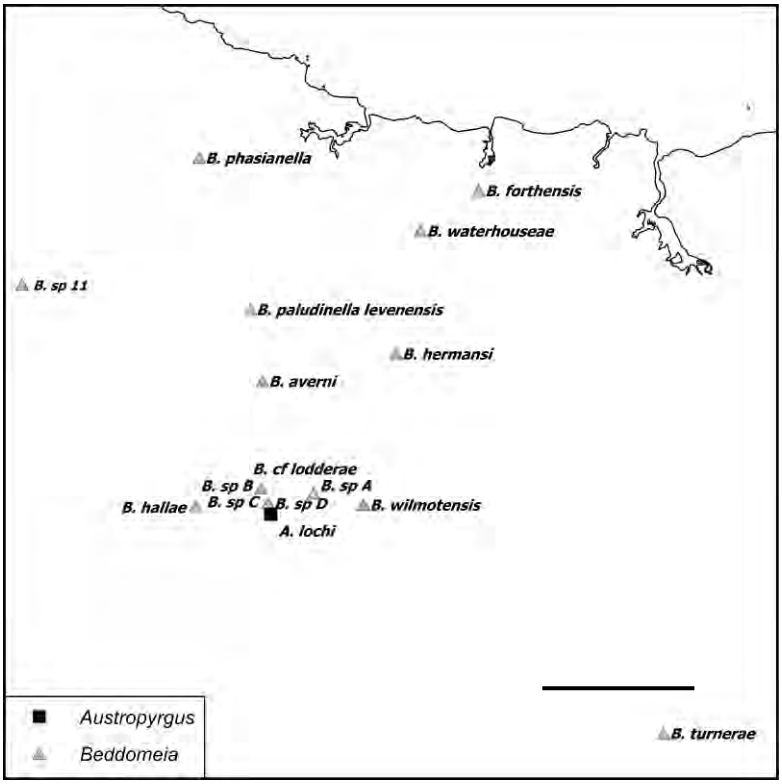


Figure 6.2*d*. Location of specimens obtained in central Tasmania. *B. sp. A – D* are morphotypes recognised in Chapter 3. Scale 10 km.

Table 6.1. Locations of taxa used in this study. Grid references in GDA.

Taxa	Location	Grid reference
Beddomeia averni	3km E of Preston, tributary of West Gawler R.	424212 5429083
Beddomeia briansmithi	Fern Ck, tributary of Carters Ck, 0.8km ESE of Forester on Conners Rd	557562 5451683
Beddomeia cf *minima*	McKenzie Rivulet on North Rd, S of Scottsdale	545912 5434533
Beddomeia forthensis	Tributary of Forth R, on S side of Geales Hill on Wilmot Rd	436542 5439933
Beddomeia fromensis	Trib of Frome R on NW side, 0.5km NE along Garibaldi Rd, Moorina	575912 5445483
Beddomeia hallae	Buttons Rivulet on South Preston Rd	420424 5421963
Beddomeia hullii#	Trib of Thirteen Mile Ck, ca. 0.8km E of Mt Cleveland Rd on Corinna Rd	362112 5407780
Beddomeia hermansi	Top end of Viking Ck, trib of Wilmot R.	431812 5430633
Beddomeia kessneri	Dip River, above falls nr Mawbanna	363512 5456133
Beddomeia krybetes	St Pauls R, ca 5km E of Royal George on Merrywood Rd	578222 5370333
Beddomeia launcestonensis	Scour hole, South Esk R., 700 m below Trevallyn Dam	507412 5410983
Beddomeia minima #	St Patricks River, Tasman Hwy	*532112 5427062
Beddomeia pallida	Headwaters of Second R, 7.5km E along Doaks Rd from Lilydale	522962 5432580
Beddomeia paludinella levenensis	Leven R at Bannons bridge	423512 5433163
Beddomeia paludinella paludinella	Hellyer R at Hellyer Gorge, Murchison Hwy, mouth of small creek, upstream from bridge.	383900 5429883
Beddomeia phasianella	Keddies Ck on Dial Rd, S of Penguin	420600 5441800
Beddomeia ronaldi	Weavers Creek, Nunamara	527812 5418483
Beddomeia tasmanica	Headwaters of Terrys Creek, Goshen	592852 5434273
Beddomeia turnerae	Trib of Minnow R, Lower Beulah Rd on NE Kenzies Hill	447062 5409023
Beddomeia waterhouseae	Small trib of Little Claytons Rivulet, off Thompson Rd, S of Ulverstone	433212 5437633
Beddomeia wilmotensis	Trib. of Wilmot R. 400 m downstream of Spellmans Bridge, Castra	429962 5422043
Beddomeia sp. A[##]	Trib. of Castra Rivulet, Nietta	427122 5422683
Beddomeia sp. B[##]	Castra Rivulet, Nietta	424512 5422123
Beddomeia sp. C[##]	Castra Rivulet, Nietta	424682 5421483
Beddomeia sp. D(1)[##]	Castra Rivulet, Nietta	424132 5422963
Beddomeia sp. D(2)[##]	Castra Rivulet, Nietta	424512 5422123
Beddomeia sp. 1[##]	Trib. of Groom River, Pyengana Saddle, Goulds Country	586012 5430333
Beddomeia sp. 2[##]	Trib. of Groom River, Pyengana Saddle, Goulds Country	586012 5430333
Beddomeia sp. 3[##]	Dead Horse Hill Ck. trib. of Groom River, Blue Tier	581112 5436363
Beddomeia sp. 4 [##]	Le Fevre Ck. trib. of Nth George R. Weldborough Pass	576692 5433883
Beddomeia sp. 5[##]	Seepage into Mt Albert Rivulet, trib. of Sth George R., at St Columba Falls	577052 5424963
Beddomeia sp. 11[@]	Trib of Blythe R on South Riana Rd	410512 5434583
Pseudotricula eberhardi#	Bauhaus Cave, Precipitous Bluff	*469812 5185583
Nanocochlea sp.#	Damper Creek, Precipitous Bluff	*467092 5185603
Austropyrgus simsonianus#	Jordan River, at Brighton	521712 5272283
Austropyrgus cf *lochi*	Castra Rivulet, Nietta	424682 5421483
Phrantela pupiformis	Trib. of 14 mile Ck, Styx Rd, Maydena	465112 5262533
Phrantela daveyensis tristis#	Trib. of Dismal Ck, trib. of Hardwood R.	*407612 5247425
Phrantela marginata#	Thirteen Mile Creek, trib of Heazlewood R., junction of Mt Cleveland & Corinna Rds.	362112 5407780
Potamopyrgus antipodarum#	M. Haase, unpublished data	**

* Obtained from Genbank, with collection grid reference information unavailable (Perez *et al*. 2005). Grid references obtained from collection localities, or converted into GDA from references in original papers (Ponder *et al.* 1993, Perez *et al.* 2005, Ponder *et al.* 2005).

** No grid reference available.

Obtained from Genbank, with collection grid reference information

Morphotypes identified in Chapter 3.

@ Undescribed species, AMS species number, AMS database

Table 6.2. List of species sequences used in 16S, CO1 and combined analyses.

Taxa	16S	CO1	Combined
Beddomeia averni	*	*	*
Beddomeia briansmithi	*	*	*
Beddomeia cf *minima*	*	*	*
Beddomeia forthensis	*	*	*
Beddomeia fromensis	*		
Beddomeia hallae	*	*	*
Beddomeia hullii#	*	*	*
Beddomeia hermansi	*	*	*
Beddomeia kessneri	*		
Beddomeia krybetes	*	*	*
Beddomeia launcestonensis	*	*	*
Beddomeia minima #	*	*	*
Beddomeia pallida	*	*	*
Beddomeia paludinella levenensis	*	*	*
Beddomeia paludinella paludinella	*	*	*
Beddomeia phasianella	*	*	*
Beddomeia ronaldi	*	*	*
Beddomeia tasmanica	*		
Beddomeia turnerae	*	*	*
Beddomeia waterhouseae	*	*	*
Beddomeia wilmotensis	*	*	*
Beddomeia sp. A##	*		
Beddomeia sp. D(1)##	*	*	*
Beddomeia sp. B##	*	*	*
Beddomeia sp. C##	*	*	*
Beddomeia sp. D(2)##	*	*	*
Beddomeia sp. 1##	*	*	*
Beddomeia sp. 2##	*		
Beddomeia sp. 3##	*	*	*
Beddomeia sp. 4 ##	*	*	*
Beddomeia sp. 5##	*	*	*
Beddomeia sp. 11@	*	*	
Pseudotricula eberhardi#	*	*	*
Nanocochlea sp.#	*		
Austropyrgus simsonianus#	*	*	*
Austropyrgus cf *lochi*	*	*	*
Phrantela pupiformis	*		
Phrantela daveyensis tristis#	*	*	*
Phrantela marginata#		*	
Potamopyrgus antipodarum#	*	*	*
Mercuria similis	*	*	*
Cincinnatia winkleyi	*	*	*
Hydbrobia acuta	*	*	*

6.3.3 Analyses

Sequences were visually aligned using BioEdit v7.0.5.2 (Hall 1999, 2001, Hall 2004), with the initial alignment conducted using ClustalW (Thompson *et al.* 1994). Alignment of 16S was done without reference to secondary structure models due to the high levels of primary sequence conservation, and is in line with similar studies such as by Lui & Hershler (2005).

Maximum likelihood (ML), neighbour-joining (NJ) and parsimony (MP) analyses for CO1, 16S and the combined datasets were performed in Paup*4.0b10 (Swofford 2002), employing MrModeltest 2.21 (Posada and Crandall 1998, Nylander 2004) to determine appropriate evolutionary models for ML heuristic searches. Phylogenetic trees were constructed in MacClade 4.03 (Maddison and Maddison 2000) and TreeviewX (Page 1996). A Bayesian analysis on the combined sequences was undertaken using MrBayes v3.1.2 (Ronquist and Huelsenbeck 2003).

Parsimony analyses were conducted using the following options: heuristic MP searches with 100 replicates, stepwise addition, uninformative characters ignored, only minimal trees were kept and gaps examined both as missing and as 5th base (separate analyses), and zero length branches were collapsed. Due to the low level of variability in the datasets, all substitutions received equal weightings. Phylogenetic trees were rooted using the outgroups identified above. Likelihood analyses were performed using the GTR+G model for 16S, while the GTR+G+I model was applied to both the CO1 and combined analyses. The HKY model (Hasegawa *et al.* 1985) of sequence evolution with gamma distributed rates was used to construct a NJ tree and to estimate transition/transversion (TS/TV) ratios and base frequencies. Bootstrapping (BS) with 1000 replications using the „fast' stepwise-addition option was used to estimate support values for internal nodes in the combined dataset ML analysis and individual gene datasets.

Bayesian analyses were run on the combined data matrix using the GTR (general time reversible i.e. variable base frequencies & symmetrical substitution matrix+I (proportion of invariable sites +G (gamma distribution, i.e. gamma distributed rate variation among sites)) model of evolution. The dataset was partitioned, with CO1 nucleotide frequencies set at the default. Metropolis-coupled Markov chain Monte Carlo (MCMCMC) simulations were conducted with two runs each containing 4 chains. Analyses employed either the default random tree option for 1,000,000 generations or 10,000,000 generations, with chains sampled at intervals of 1000 and a default burnin removing 25% of trees from the cold run. Estimated posterior probabilities (*P*

values) over 50% were calculated and represented on consensus trees, with *P* values of 95% considered evidence of significant support for specific clades (Huelsenbeck and Ronquist 2001).

6.4 Results

6.4.1 Sequence data

A combined aligned data matrix of 1113 base pairs [bp] (595 bp CO1; 518 bp 16S rDNA) was produced from the partial CO1 and 16S rDNA sequences. Average base frequencies for 16S rDNA were 31.04% A, 31.86% T, 14.46% C and 22.63% G and for CO1 were 41.36% A, 18.89% T, 24.26% C and 15.50% G. Parsimony analysis of the 16S data is shown in Figure 6.3 (42 sequences representing 41 taxa including a replicate of one taxon, 518 characters, 126 parsimony-informative and 56 parsimony-uninformative) resulting in 28 trees (390 steps, CI = 0.669 including uninformative characters). A MP analysis of the CO1 data is shown in Figure 6.4 (47 sequences including replicates of 12 taxa representing 35 taxa; 595 characters, 210 parsimony-informative, and 39 parsimony-uninformative) resulting in 72 most-parsimonious trees recovered (829 steps, CI = 0.473 including uninformative characters). The combined dataset MP analysis (33 taxa, 1113 characters, 302 parsimony-informative and 108 parsimony-uninformative) resulted in 28 most-parsimonious trees (1078 steps, CI = 0.555).

Maximum Likelihood and NJ analyses produced similar tree topologies to those obtained in the parsimony analyses, with only minor changes to topologies at some internal nodes (with low support values), therefore, only parsimony trees are reproduced here to represent topologies recovered for 16S and CO1 sequences. Results of the Bayesian analysis conducted on the combined dataset and strict consensus of the most-parsimonious trees also differed at some internal nodes, most particularly regarding the placement of *Phrantela* in the topology, therefore both are represented in Figures 6.5*a,b*.

Scatter plots of the transitions vs transversions (TI/TV) for each of the 16S, CO1 and combined datasets revealed linear relationships between the two, indicating that TI is not saturated and therefore still phylogenetically informative (not reproduced here). Transition/transversion ratios were estimated at 2.904 for 16S, 2.954 for CO1, and 2.590 for the combined dataset. As expected, the number of TI's outnumbered TV's within the *Beddomeia* and *Phrantela* while the number of TI's and TV's were found to increase with genetic distance, a result similar to those reported in Perez *et al.* (2005).

6.4.2 Phylogenetic relationships amongst the **Beddomeia** *species*

Multiple specimens were collected from the same locations and sequenced for both 16S and CO1; however, replicate 16S species sequences showed no genetic variability and replicate CO1 sequences revealed only low level genetic variability, providing limited data on local genetic variation. Only replicate CO1 sequences were included in the analyses, with the exception of one replicate 16S sequence.

Results of the combined dataset analyses were generally congruent with the individual partial-gene analyses. Parsimony, likelihood, distance and Bayesian analyses for the single and combined datasets recovered similar tree topologies for most *Beddomeia* species and morphotypes, differing only at a few weakly supported internal nodes; however, the analyses did differ regarding the nesting of *Phrantela* spp. and *Austropyrgus* spp. within *Beddomeia* clades.

Bayesian probabilities were higher than the BS values, a trend which has been reported in other papers (e.g. Cummings *et al.* 2003, Casteo *et al.* 2004, Perez *et al.* 2005) and is said to relate to an over-estimation of support (Perez *et al.* 2005). Bootstrap values obtained in the individual gene analyses showed high support for the five clades identified; similar, but not consistent groupings of species were recorded in all analyses.

The topologies recovered raise questions about the monophyly of *Beddomeia*; the data provide conflicting evidence of the relationships between *Phrantela, Austropyrgus* and *Beddomeia*. Analyses of 16S data show *A.* cf *lochi* nested in a clade with NE *Beddomeia* species (Figure 6.3) while *B. fromensis* was a sister taxon to the *Beddomeia* clades, and *Phrantela pupiformis* and *P. daveyensis tristis* nested with the east Tamar *Beddomeia* species. This relationship is not supported in the CO1 analyses, rather, *P. daveyensis* is nested with the „Tamar group' *Beddomeia*, in an otherwise monophyletic *Beddomeia*, although there is limited support for this placement.

Only the combined dataset MP, ML and NJ analyses support the monophyly of *Beddomeia*, with *P. daveyensis* recovered as sister taxon to the monophyletic *Beddomeia* clade; however, this result is not repeated in the Bayesian analysis, which shows high Bayesian probability support for nesting *Phrantela daveyensis* into the NE *Beddomeia* clade, indicating that *Beddomeia* is paraphyletic.

Nevertheless, a number of well supported clades within *Beddomeia* are recognised across all analyses, representing particular spatial or geographical relationships within the genus. The relationship between the phenetic species groups (PSG) (Ponder *et al.* 1993) and molecular phylogenetic clades for the named species used in the analyses is presented in Table 6.3 and illustrated in Figures 6.6 and 6.7, and shows some similarity between the groupings, but not all. Species occurring in the east of the state are divided into two clades; the „NE Plomley's Island' group and the „East Tamar' group, in line with the invertebrate bioregions identified by Mesibov (1996b) in which they occur. The Plomley's Island group species *B. briansmithi*, *B.* cf *minima*, *B. minima*, *B. tasmanica* and morphotypes 1 – 5 form a well supported, recognisable clade, with a Bayesian posterior probability of 100 and BS values of 93 for the combined sequences, and BS of 92 for CO1 and 76 for 16S sequences, and incorporate species from two phenetic species groups, while the East Tamar clade, which is also well supported (100% posterior probability and BS of 100) for CO1 and combined analyses, consists of two species, both of which were originally placed into PSG 2 (Table 6.3). Most of the remaining species sequenced form three well supported clades associated with the „NW far' (*B. hullii* and *B.* sp. 11), the „Castra' region (*B. averni*, *B. hallae* and morphotypes B – D), and the wider -„Tamar' area (*B. launcestonensis*, *B. krybetes*, *B. turnerae*, *B. hermansi*, *B. waterhouseae* and *B. forthensis*) (Figure 6.7). While these clades are well supported across all analyses, the precise phylogenetic relationships of *B. phasianella*, *B. wilmotensis*, and the riverine species *B. paludinella levenensis*, *B. paludinella paludinella* and *B. kessneri* as well as morphotype A, within these clades are unresolved, despite showing strong northwestern or Castra group associations in most analyses. In addition, one NE *Beddomeia* species (*B. fromensis*) (location shown in Figure 6.6) was revealed as a sister taxon to *Beddomeia* clades in the 16S analyses, although this might be a reflection of the quality of the sequence, or a confusion of samples *A.* cf *lochi* and *B. fromensis*, rather than the true phylogenetic relationship. This sequence displayed a weak PCR product signal and was re-replicated, which may also have introduced contaminants. Therefore the taxonomic relationship of *B. fromensis* to the other *Beddomeia* should be considered doubtful until further sequences can be obtained.

Species validation

Sequence divergence as measured by pairwise distances between genera, were calculated and are presented in Table 6.4. Interspecific distances (mean character differences between the *Beddomeia*) ranged between 0 – 0.079 in 16S, whereas intergeneric (between genera) distances ranged from 0.048 to 0.088 for *Phrantela* and *Beddomeia* to between 0.124 – 0.163 for outgroup genera, excluding one *Austropyrgus* species. Catchment specific *Beddomeia* morphotypes displayed smaller pairwise distances within the interspecific *Beddomeia* range, or

showed similarities with named species, revealing uncertainty of the status of some species and several morphotypes using 16S sequences (Table 6.5). The mean number of character differences was also low between a several pairs of *Beddomeia* species: *B. ronaldi – B. pallida*, *B. briansmithi – B. cf minima*, and *B. krybetes* - *B. launcestonensis* which are not catchment related, suggesting minimal 16S support for the current naming of some *Beddomeia* species. Pairwise distances for some *Phrantela – Beddomeia* spp. show < 5% variation from *Beddomeia*, suggesting that insufficient character differences exist between *Beddomeia* and some *Phrantela* species to warrant generic separation. However, differences of < 5% were only recorded against four *Beddomeia* species with *P. daveyensis tristis* and once for *P. pupiformis*, all other pairs showing over 5% character differences, thus supporting genus level separation.

Table 6.3. The relationship between Phenetic species groups and Molecular phylogenetic clades for named *Beddomeia* species in this study.

Species	Phenetic Species Group number*	Phenetic species group	Molecular phylogenetic clade
B. averni	4c	*B. lodderae* subgroup	Castra
B. hallae	4c	*B. lodderae* subgroup	Castra
B. phasianella	7	*B. phasianella*	Castra (Wilmot)
B. wilmotensis	1	*B. launcestonensis*	Castra (Wilmot)
B. pallida	2d	*B. tasmanica*	East Tamar Break
B. ronaldi	2d	*B. tasmanica*	East Tamar Break
B. minima	4b	*B. minima* subgroup	NE – Plomleys Is
B. cf *minima*	4b	*B. minima* subgroup	NE – Plomleys Is
B. fromensis	4b	*B. minima* subgroup	NE – Plomleys Is
B. briansmithi	4b	*B. minima* subgroup	NE – Plomleys Is
B. tasmanica (Woods)	2a	*B. tasmanica*	NE – Plomleys Is
B. hullii	4a	*B. hullii* subgroup	NW (Far)
B. kessneri	2b	*B. tasmanica*	NW (River)
B. palludinella palludinella	3	*B. paludinella*	NW (River)
B. paludinella levenensis	3	*B. paludinella*	NW (River)
B. launcestonensis	1	*B. launcestonensis*	Tamar
B. krybetes	1	*B. launcestonensis*	Tamar
B. waterhouseae	4c	*B. lodderae* subgroup	Tamar
B. hermansi	4c	*B. lodderae* subgroup	Tamar
B. forthensis	4c	*B. lodderae* subgroup	Tamar
B. turnerae	4c	*B. lodderae* subgroup	Tamar

* From Ponder *et al.* (1993).

Table 6.4. 16S Pairwise distances between taxa (mean character differences – adjusted for missing data) for 16S sequences for *Phrantela* (2 species), *Beddomeia* (31 species, subspecies and morphotypes), *Austropyrgus* (2 species), one each of *Nanocochlea* and *Potamopyrgus*, and the three additional outgroup species.

	Phrantela	*Beddomeia*	*Pseudotricula*	*Nanocochlea*	*Potamopyrgus*	*Austropyrgus*	*Mercuria*	*Hydrobia*	*Cincinnatia*
Phrantela	0.021	0.048 – 0.088	0.132 – 0.134	0.130 – 0.131	0.160 – 0.169	0.059 – 0.157	0.142 – 0.144	0.155	0.142 – 0.146
Beddomeia		0 – 0.079	0.125 - 0.159	0.126 – 0.152	0.139 – 0.175	0.008 – 0.161	0.124 – 0.163	0.133 – 0.162	0.126 – 0.158
Pseudotricula			0	0.002	0.059	0.058 - 0.126	0.127	0.151	0.130
Nanocochlea				0	0.061	0.059 - 0.122	0.126	0.147	0.132
Potamopyrgus					0	0.053 - 0.143	0.124	0.152	0.116
Austropyrgus						0 – 0.127	0.129 – 0.138	0.133 – 0.157	0.123 – 0.126
Mercuria							0	0.714	0.058
Hydrobia								0	0.067
Cincinnatia									0

Table 6.5. 16S Pairwise distances (mean character differences – adjusted for missing data) for 16S sequences, between *Beddomeia* morphotypes and some species in close proximity. (*B.* sp. D[1] and sp D[2] were used to demonstrate variability in this morphotype)

Species pair	Mean character difference	No. of character differences
B. ronaldi – B. pallida	0.018	9
B. sp. 1 – *B.* sp. 3	0	0
B. sp. 4 – *B.* sp. 5	0.029	15
B. tasmanica – B. sp. 2	0.017	8
B. tasmanica – B. sp. 5	0.046	23
B. briansmithi – B. cf *minima*	0	0
B. briansmithi – B. sp. 1	0.004	2
B. briansmithi – B. sp. 3	0.006	3
B. krybetes – B. launcestonensis	0.006	3
B. sp. A – *B.* sp. B	0.04	20
B. sp. A – *B. wilmotensis*	0.01	5
B. sp. B – *B.* sp. D[1]	0.015	8
B. sp. C – *B.* sp. A	0.039	18
B. sp. C – *B.* sp. B	0.032	15
B. sp. C – *B.* sp. D[1]	0.022	10
B. sp. D[1] – *B.* sp. A	0.026	13
B. sp. D[1] – *B.* sp. D[2]	0.004	2
B. hallae – B. sp. D[1]	0.002	1
B. hallae – B. sp. D[2]	0.004	2
B. hallae – B. sp. B	0.014	7

6.4.3 Other hydrobiids

Sequences for only two Tasmanian species of *Austropyrgus* were obtained for this study. Using these to analyse the relationship between *Beddomeia* and *Austropyrgus* reveals some taxonomic uncertainty, with topologies differing for the individual and combined datasets. MP, ML and NJ analyses on the CO1 dataset nest both *Austropyrgus* species with *Potamopyrgus antipodarum*, whereas the 16S analyses nest *A.* cf *lochi* within the Plomley's Island *Beddomeia* clade and *A. simsonianus* with the outgroup, which suggests that *Austropyrgus* is not monophyletic. Both NJ and ML analyses on the combined dataset suggest *Austropyrgus* is a sister group to *Beddomeia*; however, the Bayesian analyses (posterior probabilities of 99%) support an *Austropyrgus – Pseudotricula* clade, sister group to *Beddomeia*, while MP analysis of the combined dataset indicates that *A.* cf *lochi* is a „sister taxon' to the *Beddomeia* clade, and *Phrantela daveyensis tristis* as well as to the clade containing *A. simsonianus* and *P. eberhardi*. As with *B. fromensis*, some uncertainty about the origin of the 16S sample of *A.* cf *lochi* remains, therefore, the

taxonomic relationship of *A.* cf *lochi* to the other *Austropyrgus* should be considered doubtful until further sequences can be obtained.

Of the three *Phrantela* species included in the analyses, combined sequences could only be obtained for one species, *P. daveyensis tristis*, with a different combination of two *Phrantela* species included for each of the single gene analyses. Neither 16S or CO1 support monophyletic origins for *Phrantela*. In the CO1 analysis *Phrantela daveyensis tristis* is nested within the *Beddomeia* Tamar group and *P. marginata* forms a sister clade to *Beddomeia*, while in the 16S sequence analyses *Phrantela* species, *P. daveyensis tristis* and *P. pupiformis* form a well supported clade with the East Tamar and far NW *Beddomeia* species. Bayesian analysis of the combined datasets also nests *P. daveyensis tristis* with the East Tamar clade, however, the MP and ML analyses of the combined data place this species as a sister taxon to *Beddomeia*.

The remaining two Tasmanian genera, *Pseudotricula* and *Nanocochlea*, were included in the 16S analysis, which found the genera are closely related. No CO1 sequence was available for *Nanocochlea*; however, *Pseudotricula* sequences were obtained for 16S and CO1, with 100% posterior probability support for its relationship with *Austropyrgus simsonianus* provided in the Bayesian analyses.

Nanocochlea, *Pseudotricula* and *Potamopyrgus* were assessed as suitable for use as outgroups by comparisons of topologies recovered when incorporating the more distantly related species: *Cincinnatia winkleyi*, *Hydrobia acuta* and *Mercuria similis*. Similar relationships were observed in the topologies recovered using all six species, versus only the latter three species, as outgroups. *Cincinnatia winkleyi*, *H. acuta* and *M. similis* were retained in the analyses for comparative reasons only. As expected, *C. winkleyi*, *H. acuta* and *M. similis* showed the most distant relationships to *Beddomeia* in each of the analyses, while the relationship between *Potamopyrgus* and *Austropyrgus* raises questions about possible historical connections.

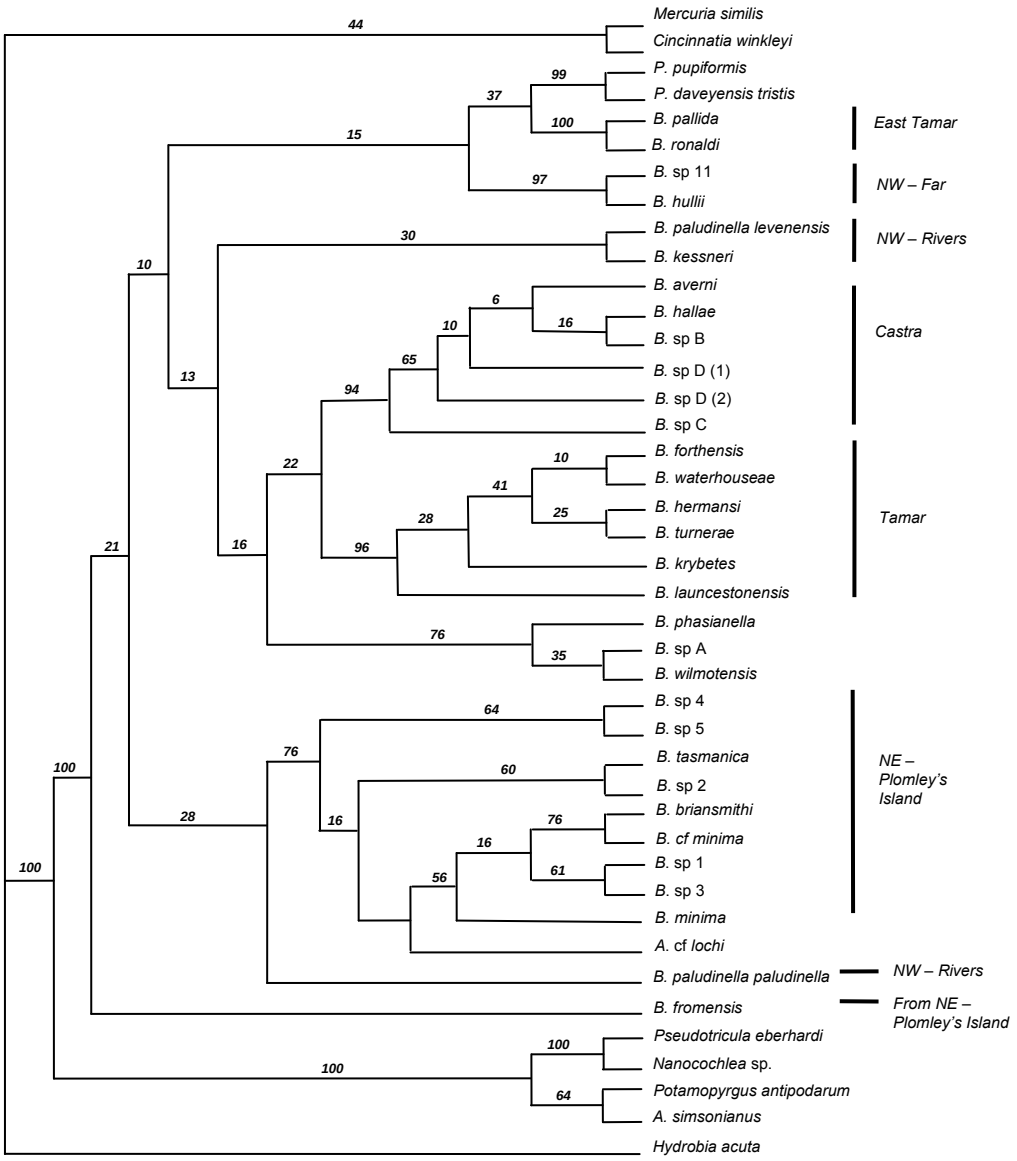


Figure 6.3. 16S MP Strict Consensus of 28 equally parsimonious trees rooted using *C. winkleyi*, *M. similis* and *H. acuta.* CI = 0.669, RI = 0.805, RC = 0.539, HI = 0.331 G-fit = -101.250. Tree length 390. Figures above lines are bootstrap values. Figures in brackets represent replicate sequences.

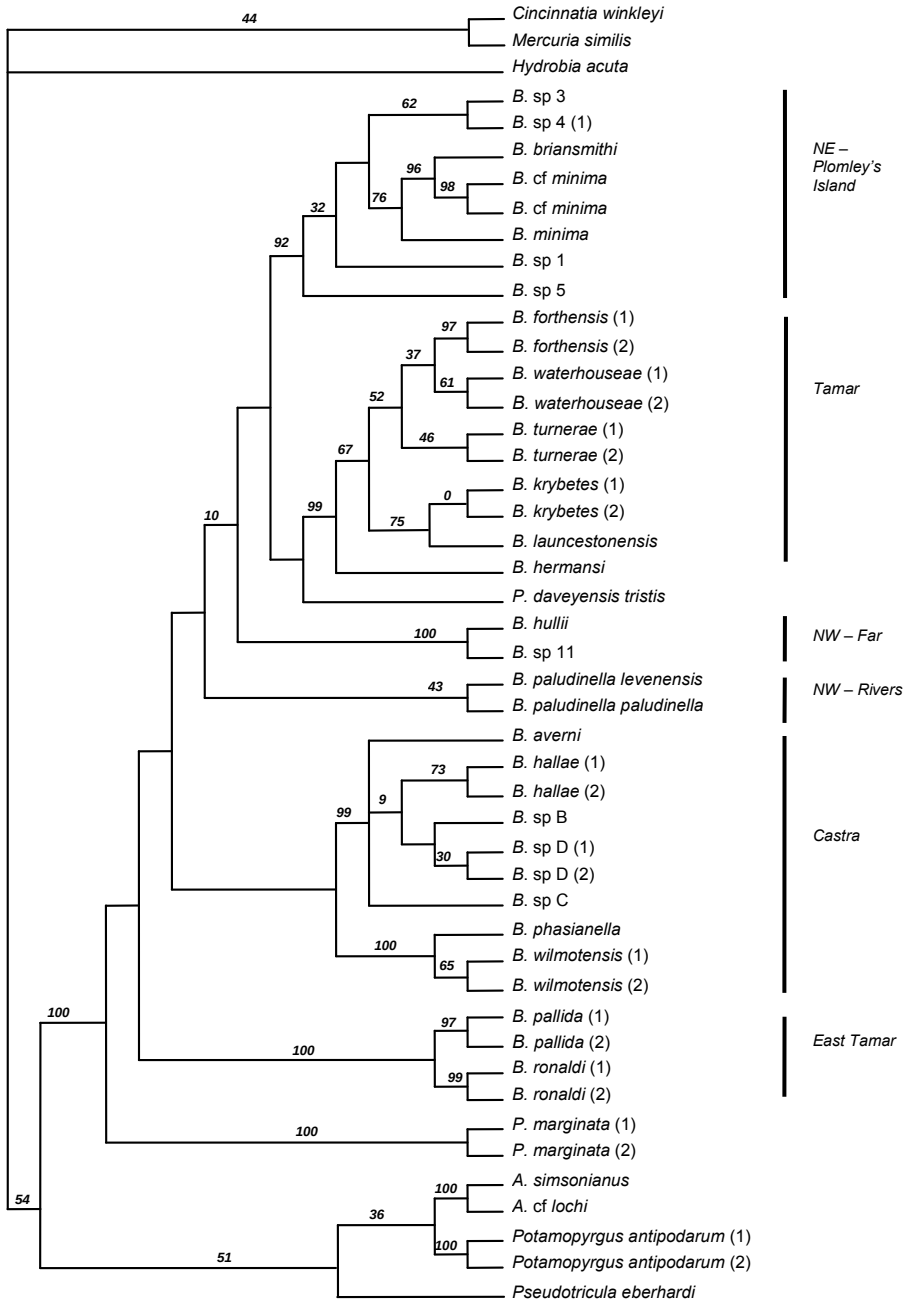


Figure 6.4. MP – 1 of 72 equally parsimonious trees for the CO1 sequence, rooted using *C. winkleyi*, *M. similis* and *H. acuta* as outgroups. CI = 0.473, RI = 0.740, RC = 0.350, HI = 0.527 G-fit = -136.320. Tree length 829. Figures above lines are bootstrap values. Figures in brackets represent replicate sequences.

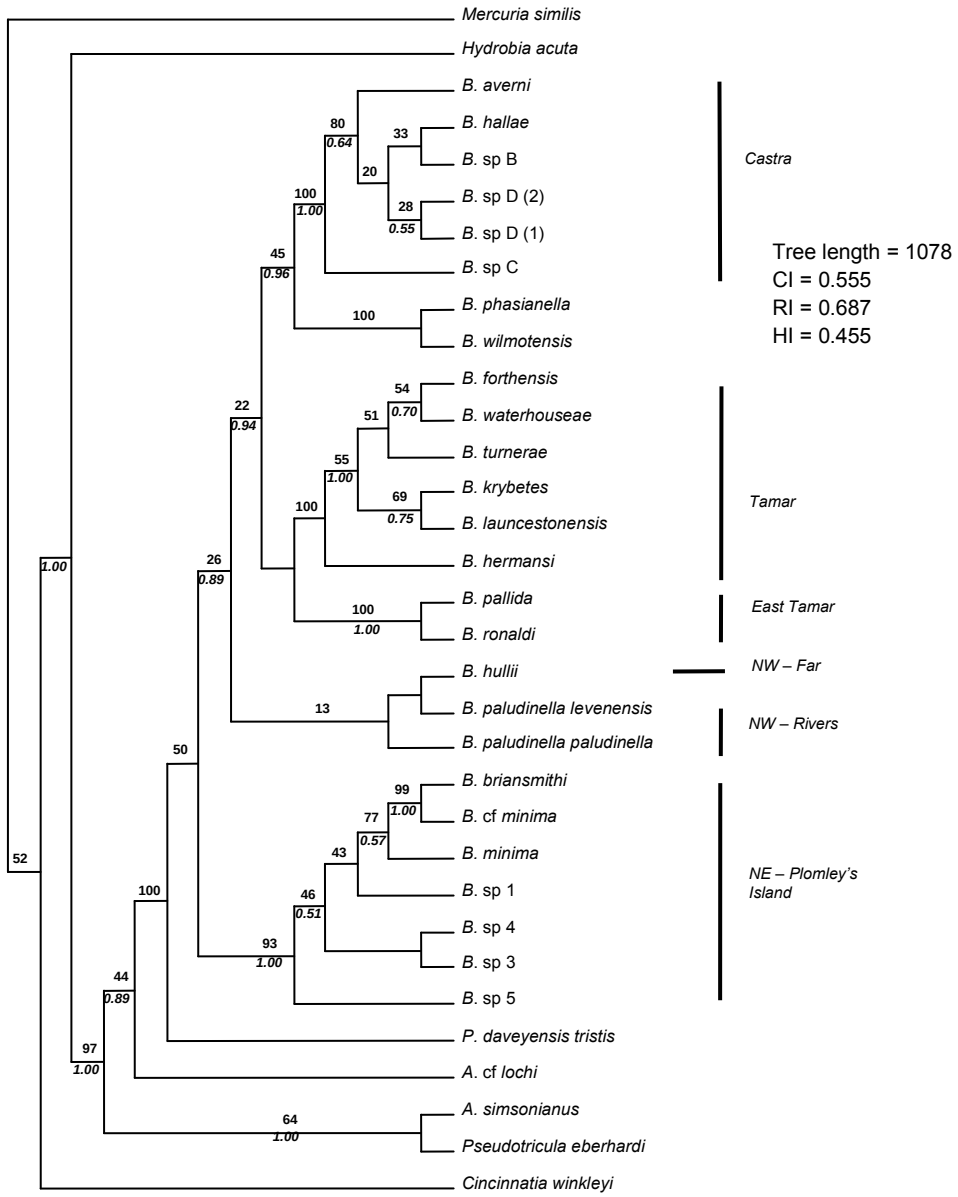


Figure 6.5*a*. MP – 1 of 28 most-parsimonious trees for the Combined sequence. Trees rooted using *C. winkleyi*, *M. similis* and *H. acuta* as outgroups. Figures above and below lines are bootstrap values and Bayesian posterior probabilities (0 – 1) respectively. Figures in brackets represent replicate sequences.

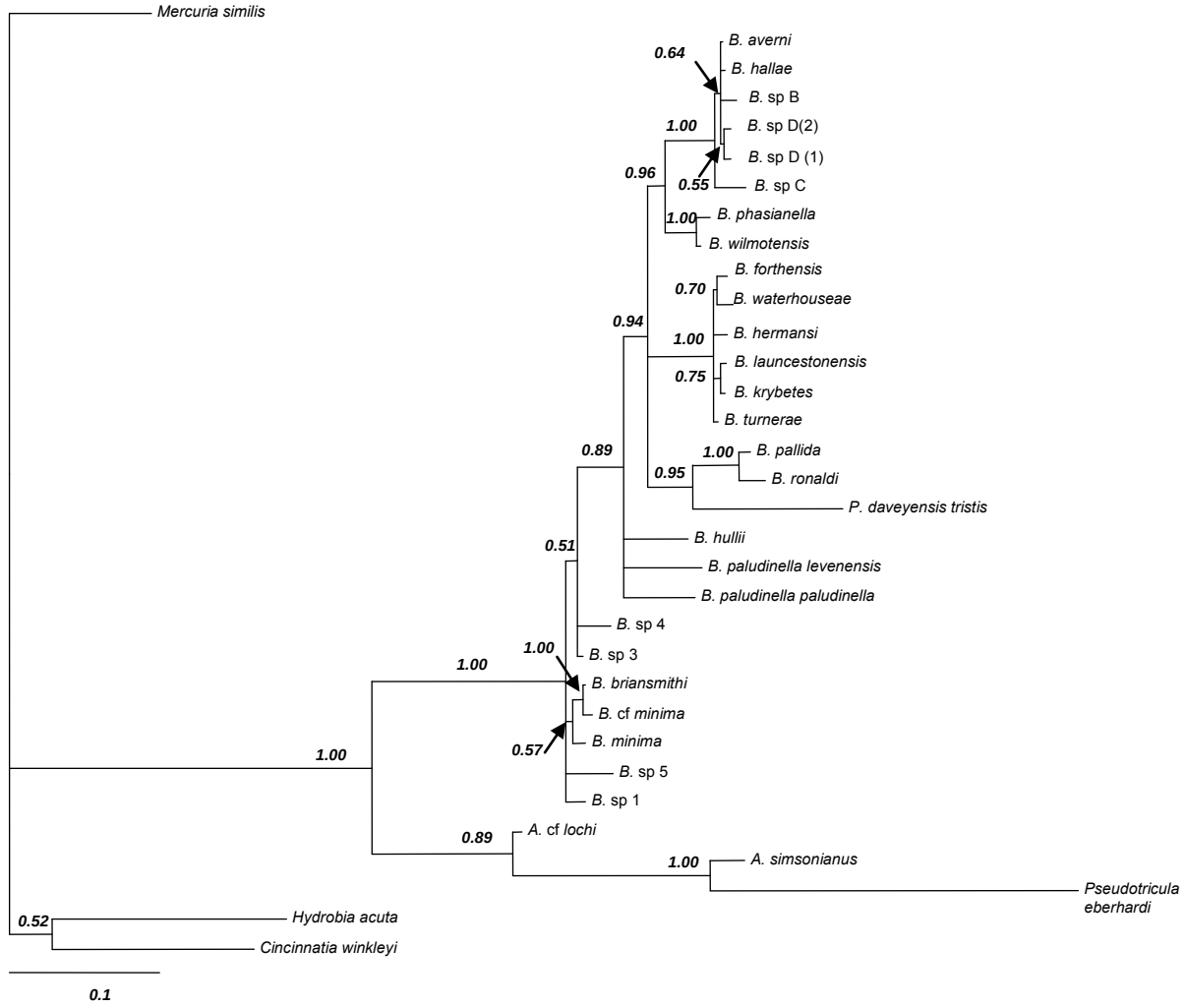


Figure 6.5*b*. Bayesian consensus tree showing Bayesian posterior probability values (0 – 1) indicating Clade credibility. Figures in brackets represent replicate sequences.

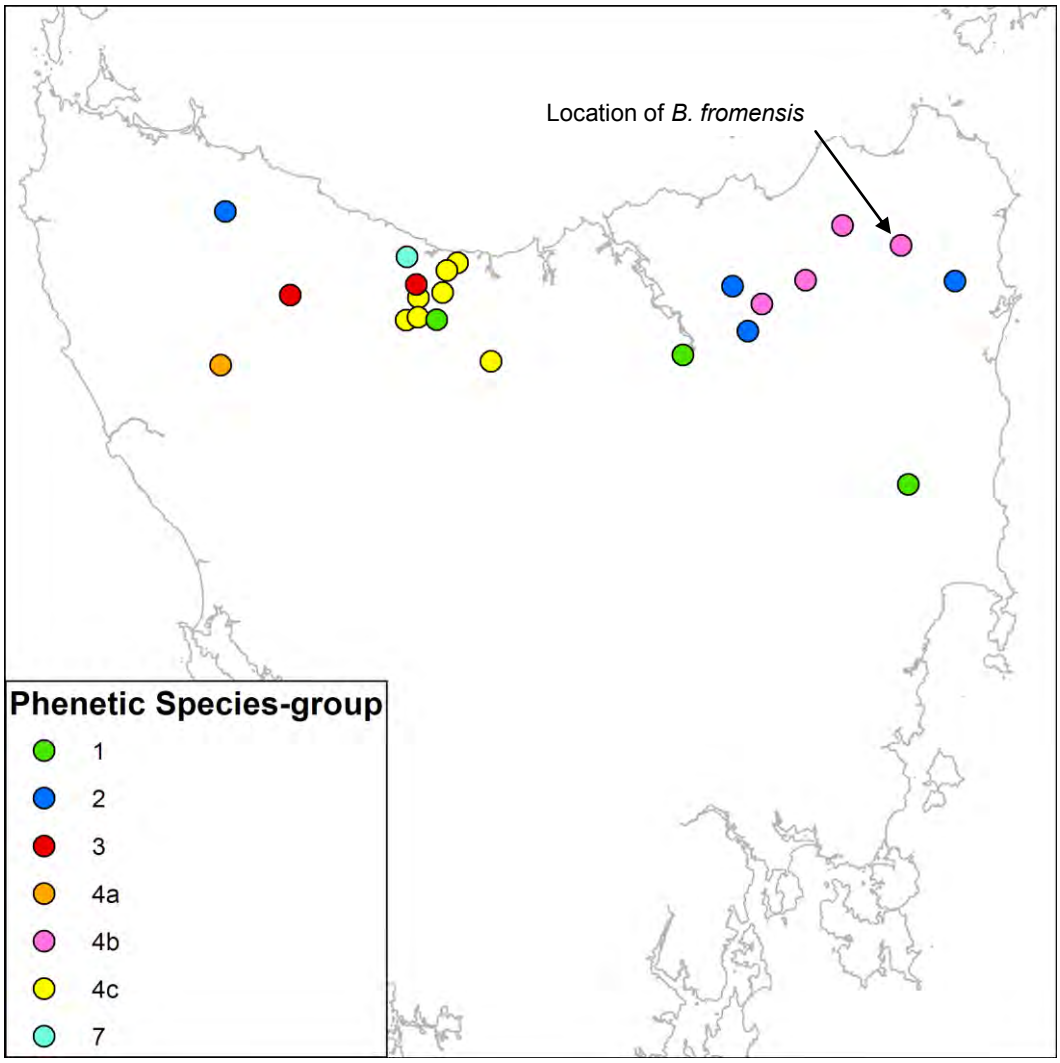


Figure 6.6. Phenetic species groupings of species used in the current study from Ponder *et al* (1993).

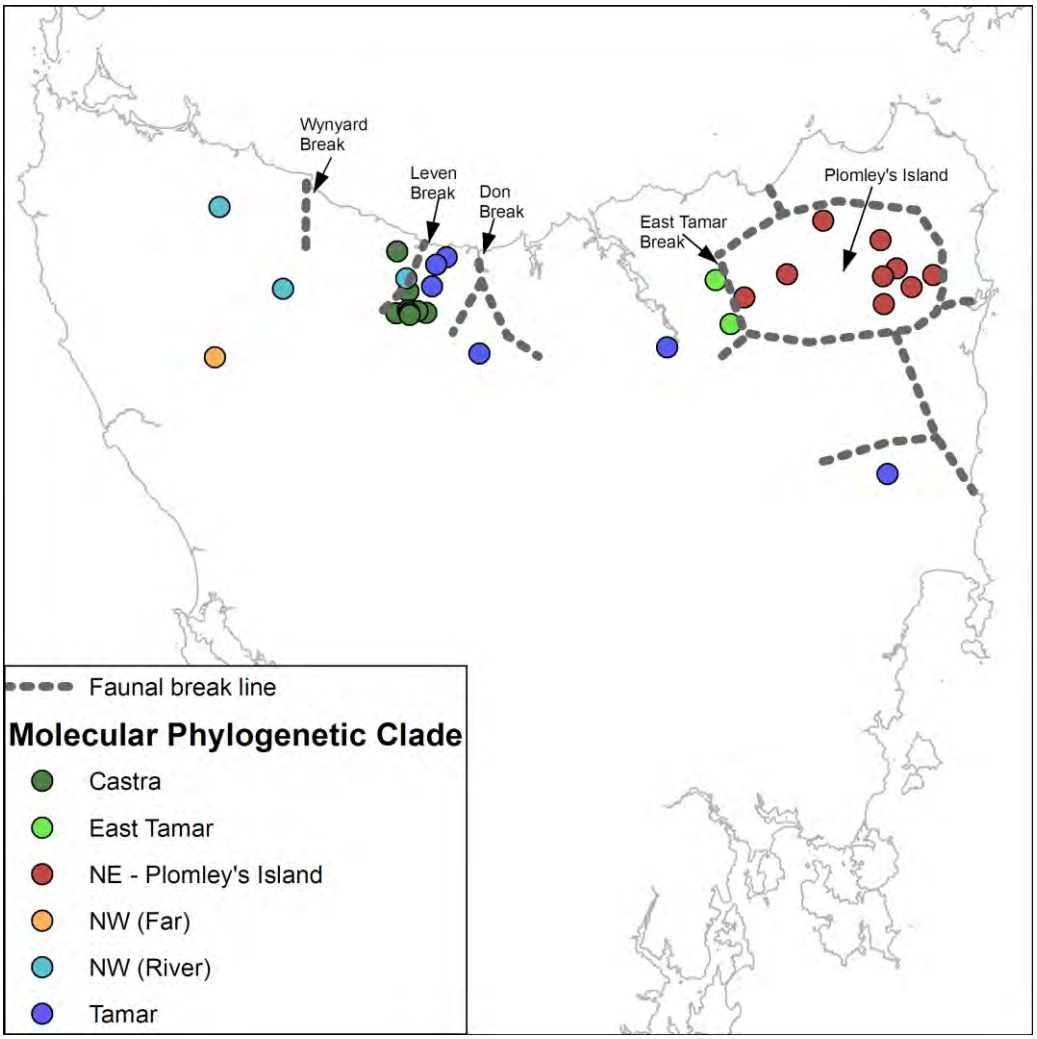


Figure 6.7. Distribution of species within *Beddomeia* molecular species groupings in relation to invertebrate faunal breaks identified in Mesibov (1996).

6.5 Discussion

Monophyly of *Beddomeia*

The topologies recovered for the 16S, CO1 and combined datasets suggest continuing uncertainty about taxonomy of Tasmania's endemic hydrobiids, specifically the monophyly of *Beddomeia* in relation to its anatomically closest genus, *Phrantela* and to *Austropyrgus.* While parsimony, distance and likelihood analyses of the combined dataset indicate monophyly of *Beddomeia* (Figure 6.5*a*), this is based on only one *Phrantela* sequence. In the topology recovered for the combined Maximum Parsimony (MP) *P. daveyensis tristis* is revealed as sister-taxa to *Beddomeia*, whereas the high posterior probability recovered in the Bayesian analysis supports inclusion of *Phrantela daveyensis* with the East Tamar *Beddomeia* clade,

(Figure 6.5*b*). Further support for a paraphyletic *Beddomeia* is revealed in the topologies recovered in the individual 16S and CO1 analyses. Analyses of CO1 sequence finds *P. daveyensis* more closely related to the Tamar group species (Figure 6.4) and *P. marginata* as a sister taxon to the *Beddomeia* clade, while for 16S, *P. pupiformis* and *P. daveyensis tristis* are closely related to the East Tamar species (Figure 6.3).

The nesting of *A*. cf *lochi* within the NE Plomley's Island *Beddomeia* group, the close association of *P. daveyensis* and *P. pupiformis* to the East Tamar group and the placement of *B. fromensis* as a sister taxon to *Beddomeia* in the 16S analyses all question the monophyly of *Beddomeia*. In the case of *A.* cf *lochi*, however, the topologies recovered from the other analyses do support monophyly of *Beddomeia*, while the lack of a CO1 sequence for *B. fromensis* prevents a valid assessment of its relationship with other *Beddomeia*, particularly as the re-amplification of the *B. fromensis* gene fragment and re-sequencing it may have resulted in a poor quality sequence due to possible contamination.

Previous cladistic analysis of a subset of anatomical characteristics undertaken by Ponder *et al.* (1993) established the monophyly of the generic groups (*Beddomeia*, *Phrantela*, *Nanocochlea* and *Victodrobia*) within the *Beddomeia* complex; however, it is acknowledged that many alternative phylogenies could be produced, dependent on the inclusion or exclusion of characters and the assumptions made. Despite the conclusion that *Phrantela* and *Beddomeia* were monophyletic, the difficulty in differentiating between these genera was also recognised (Ponder *et al.* 1993), the authors noting that *Phrantela* appears to be more plesiomorphic in some anatomical characters. Of all the characters measured, the size and extent of the female bursa copulatrix, and the position of its duct, emerging from the ventro-posterior corner, and characteristics of the male and female genital ducts and female opening were recognised as the most useful characters in separating members of *Phrantela* from *Beddomeia*.

One possible reason for the uncertainty about the molecular phylogenetic topologies recovered is that, despite the inclusion of several *Phrantela* species in the individual analyses, only one *Phrantela* sequence was available for the combined dataset, and this was obtained from the Perez *et al.* (2005) study, limiting the value of the findings, particularly where it remains the only sequence currently available for such analyses. Some doubt concerning the origin of the *P. daveyensis tristis* and *B. launcestonensis* sequences obtained from Genbank surfaced during the preliminary molecular analyses, where the *B. launcestonensis* more closely resembled the *Phrantela* sequences than it did the *B. launcestonensis* sequenced in this study. Consequently, the Genbank *B. launcestonensis* sequence was removed from the analyses while the *Phrantela*

was retained as it remains the only „combined' sequence available. It is possible that the sequences for *B. launcestonensis* and *P. daveyensis tristis* recovered by Perez *et al.* (2005) were mislabelled, this is supported by the nesting of the latter within the Tamar group (of which *B. laucestonensis* is part) in the CO1 analyses, but does not explain the relationship between *Phrantela* spp. and the East Tamar *Beddomeia* spp. identified in the 16S analyses. The topology recovered from the Bayesian analysis is also questioned by the placement of the *Phrantela daveyensis tristis* in the remaining analyses. As a result, without further „combined' sequences becoming available for *Phrantela*, the inclusion of *P. daveyensis tristis* within the *Beddomeia* clade is questionable. Therefore, in the absence of additional supporting evidence to the contrary, it is proposed that *Beddomeia* still be considered monophyletic.

Status of species

Limited numbers of mutations were detected within the *Beddomeia* species in the more conservative 16S gene region. Mean character differences between *Beddomeia* species were low (0 – 0.079), the more geographically close species displaying the smallest differences (Table 6.4). While the number of character differences suggests that the pairs *B. ronaldi* - *B. pallida*, *B. briansmithi* - *B.* cf *minima*, and *B. krybetes* - *B. launcestonensis* are very similar and they also display similar external shell morphologies, anatomical features are incongruent, indicating that they should be regarded as separate species-level taxa.

Although morphotypes have been included in these analyses to assist in determining speciation within the catchments studied in Chapter 3, it is unfortunate that the gene sequences for both genes of several were unobtainable. For those obtained, analyses indicate only a minor level of differentiation is occurring within the two catchments studied, including between recognised species and most morphotypes. Bayesian clade credibility values for clades containing the morphotypes were 100%, however, there was less support for some individual morphotypes within these clades, including only 51% Bayesian probability support, and BS of 46, for the separation of morphotypes 3 and 4 from *B. briansmithi* and *B.* cf *minima* in the Plomley's Island clade in the combined analyses and, despite a slightly higher 64% support (BS of 80) for a separation of clade containing morphotypes B and D, a low BS value of 20 supported the separation of morphotype D from morph B and *B. hallae*.

Castra Rivulet morphotypes

16S rDNA evidence provides support for the conclusion that morphotype A is closely related to *B. wilmotensis,* with a BS value of only 35 separating the sequences; unfortunately, no CO1 sequence was available. Anatomical comparisons with *B. inflata* and *B. wilmotensis* conducted

in Chapter 5 suggest less support for morphotype A being a variant of *Beddomeia wilmotensis*, but sequences for *B. inflata* were not obtained for this study, without which the true nature of the relationship between *B. wilmotensis*, *B. inflata* and morphotype A remains uncertain.

The relationship between morphotypes B and D also remains unresolved, as 16S analyses suggest a more distant relationship than does the CO1 gene region data, the latter indicating morphotypes B and D are closely related, but distinct, while 16S identifies differences existing not only with morphotype B, but also between the replicates of morph D. The combined sequence analyses also support a close relationship between the two morphs, but more strongly aligns morphotype B with *B. hallae*. Evidence from anatomical and phylogenetic investigations in this thesis provides tangible support for a close relationship between morphotype B and *B. hallae*, although differences exist in the anatomical characters, suggesting that there has been sufficient spatial or temporal separation to allow mutations to arise. Conversely, while it is clear that morphotypes B and D are closely related, the exact nature is less clear. Without confirmation of the existence females of morphotype D, the relationship between these morphs remains unresolved.

Shell morphology of morphotype C suggests that this morph is unlike others from the Castra catchment. DNA evidence appears to substantiate this observation, indicating a close relationship with other Castra morphs, but sufficient mutational differences exist (e.g. between 10 and 18 in 16S) to consider morphotype C to be a separate species, particularly if we support the topology of the named species *B. ronaldi*, *B. pallida*, *B. krybetes*, *B. launcestonensis*, *B. briansmithi* and *B.* cf *minima*.

Groom River catchment morphotypes

Anatomical comparisons of the Groom River morphotypes (Chapter 5) suggests morphotypes 1, 2 and 6 are likely to be variants of the same species, *Beddomeia tasmanica*. However, evidence from the 16S sequence more closely relates one morphotype (morph 2), with *Beddomeia tasmanica* than morphs 1 and 6. This is unsurprising as the DNA for *B. tasmanica* was sequenced from this morph. Unfortunately, the relationship could not be explored further using CO1, as no clear sequence for either morph 2 or *B. tasmanica* was obtainable. Unlike the relationship suggested by the morphological evidence (Chapter 5), there is some genetic support from the 16S gene for combining morphotypes 1 and 3, and also closely relating these to *B. briansmithi* and *B*. cf *minima*. However, the CO1 gene region and the combined sequences do not support the 16S topology and separate morphotypes 1 and 3, only loosely associating them with *B. briansmithi* and *B*. cf *minima*.

Anatomically, morphotype 3 differs most of the four Groom River morphs, sharing characters with *B. briansmithi*, *B. fromensis* and *B.* cf *minima*, therefore it is unclear why morphotype 1 should more closely associate with *B. briansmithi* and *B.* cf *minima* than morph 3. Evidence from the pairwise distances in the 16S gene region (Table 6.4) reveals that morphotypes 1 and 3 have no character differences, accounting for the topology recovered for this gene, and suggesting an error in specimen identification prior to DNA extraction. However, similar topologies were not recovered in the CO1 or combined sequence trees.

Morphotypes 4 and 5 were included in this study to obtain baseline information on genetic variation at a larger landscape level. These morphs closely resemble *B. tasmanica*, (visual assessment of shell), although morph 5 is significantly smaller and both come from locations in the greater George River, an adjacent catchment to the south of Groom River which merge, via Ransom River, approximately 6 km downstream of site GC14 (Chapter 3). As expected, both morphotypes nested with the Groom River morphotypes in the NE Plomley's Island clade and there remain some unanswered questions concerning the varying relationships between morphotypes 3, 4 and 5 in the 16S and CO1 topologies; however, based on the topologies recovered for 16S and CO1, morphs 4 and 5 are likely to be new species. Anatomical examination of these two morphs has not been conducted as they occur outside the main focus of Chapter 3, and will need to be conducted to confirm the status of these morphotypes.

Other hydrobiids

Uncertainty about the *Phrantela – Beddomeia* phylogeny was first raised by Perez *et al.* (2005), so the results of the current study merely further the debate. Only additional sequencing of *Phrantela* species will be required to resolve this issue. The results presented here also question the relationship between *Austropyrgus* and *Beddomeia*, since the topology remain unresolved with *A*. cf *lochi* nesting within the ‚north-eastern group' in the 16S analyses. It seems likely that conclusions drawn from datasets containing limited numbers of *Phrantela* or *Austropyrgus* species is risky, particularly where single-species or single-specimens are used, as is the case for both genera in at least some of the analyses presented. Perez *et al.* (2005) first examined the relationship between eight *Austropyrgus* and a small number of *Beddomeia* species while investigating the phylogeny of hydrobiids of the Great Artesian Basin, finding that the genera separated into two well supported clades. However, *Austropyrgus* is a large genus, containing at least 76 species spread across south-eastern Australia (Clark *et al.* 2003) and only one *Austropyrgus* used in the Perez *et al.* (2005) analysis was endemic to Tasmania. A closer examination of the Tasmanian species may uncover a different relationship.

Biogeography and clades of *Beddomeia*

The Tasmanian landmass presents a high degree of variability in its geology and climate, influencing the development of vegetation communities including alpine moorlands, cool temperate rainforest, sclerophyll forests and coastal heathlands. Climatic conditions are strongly affected by the variability in the landforms, the presence of the central mountain region, the Great Western Tiers and strong westerly air currents, bringing higher annual rainfalls to the west and northwest coasts and to the higher landforms of the northeast (Mesibov 1996a, Reid *et al.* 1999). Variation in geology and historical events such as glaciation across the island also influence the pattern of vegetation cover, and consequently the biota display strong biogeographical associations.

Tylers' line (e.g. Sheil *et al.* 1989, Mesibov 1996b), an imaginary line running more or less diagonally from far northwest Tasmania across the central highlands through Derwent Bridge and Tarraleah to a site immediately south of Hobart, is the best known example of biogeographic patterning. Fine scale sampling of patterns of terrestrial invertebrate distribution has lead to the recognition of a number of distinct „invertebrate faunal breaks' across Tasmania (Mesibov 1994, 1996b) which have strong affiliations with the landscape or geological history. Although 12 such breaks have been identified, the „Plomley's Island' faunal break in the northeast is easily the most recognisable as it coincides with the highlands of the northeast (Mesibov 1994, 1996b; Figure 6.7).

Within *Beddomeia*, patterns of close species relationships are revealed by the five well-supported clades, the most apparent division occurring between the eastern and western Tasmanian species, with the divide being the Tamar valley catchment (Figure 6.7).
Taxa of the eastern group are further segregated into two clades with distribution patterns corresponding to recognised invertebrate faunal breaks: „Plomley's Island' and the „East Tamar break' (Mesibov 1996b). The far north-eastern group, associated with Plomley's Island (Mesibov 1994), separates from the East Tamar group, which is associated with a 20 km wide band (East Tamar Break) running along the eastern edge of the Tamar River (Mesibov 1996b) containing streams flowing west into the Tamar from Plomley's island (Figure 6.7). The East Tamar group comprises two species which show limited 16S and CO1 genetic differentiation and which report some similar morphological characteristics, including having the most depressed shells of species within the genus (width/length ratio 1.0 – 1.5), being of similar small size (1.2 – 1.9 mm), wide umbilicus and having a strongly indented dorsal edge on the central teeth, although other anatomical features do deviate (Ponder *et al.* 1993, Ponder 1996). The Plomley's Island clade contains species from two phenetic species-groups (PSGs) (Table 6.3),

thus display a varied set of morphological characteristics. While one member of this phylogenetic clade is small (1.7 – 2.1 mm), has an umbilicus that is narrow to moderate, and a broadly ovate with spire about equal to width (width/length ratio 0.8 – 1.0), the majority of species in the clade are more broadly ovate conic (width/length ratio 0.7 – 0.9) with convex periphery and medium to closed umbilicus, differing in features including umbilicus width, number of cusps on inner marginal teeth of radula, number of ctenidial filaments and position of opening of female genital system.

The western taxa separate into three clades that also reflect bioregional associations, although support for some species clades varies depending on the analysis method. Associations between the clades and faunal breaks in the west is not as defined as for the eastern species, with a loss of distinction between the Castra and Tamar groups in the vicinity of the lower Forth region, along the Leven break (Figure 6.7). The NW – Far clade, consisting of *B. hullii* and *B.* sp. 11, is highly supported in most analyses, or else nested with the NW - River species, *B. paludinella levenensis* and *B. paludinella paludinella*, from the same region. Its association with the Wynyard Break is only loose; however, this clade sometimes incorporates species from east and west of this poorly resolved break, including species from the Leven and Blythe Rivers, which fall between the Leven and Wynyard breaks, but which are nonetheless geographically close (Figure 6.7). Although spatially connected, species within the NW clade (including both NW - Far and NW - River species) express different morphological traits and are found in three PSGs (Table 6.3). The one member from PSG 2b is an ovate species, shell length to 2.8 mm, umbilicus medium, with width/length ratio approximating 0.8 – 0.9. The members of the NW clade are associated with PSG 3 and are relatively large (2.8 – 5.6 mm), with depressed spires, and a thickened inner lip, whereas the species from PSG 4a is small to moderate in size (2.1 – 3.3 mm), has a broadly conic to ovate conic shell, with smaller width to length ratio (0.6 – 0.8), a small to closed umbilicus and rounded periphery.

The remaining two clades contain species groups with partially overlapping distributions in the vicinity of the southern division in the Don Break (Figure 6.7), although in general, the clades remain faithful to the Don Break location. The Castra group contains a number of species and morphotypes, occurring within a 6 km^2 area. Differentiation between the Castra morphotypes is limited to a few base pair changes in some morphs, insufficient to warrant species separation (Table 6.4). Three core „named' species are members of the Castra clade and are associated with one PSG, in common with many of the Tamar group species (Table 6.3). Members of this group have an overall similarity in shell morphology, have generally conical to broadly conic (width/length ratio 0.6 – 0.8) shells, ranging in size (2.1 – 3.7 mm), with a small to closed

umbilicus and a rounded to subangular periphery. The Castra species differ from the Tamar PSG 4c group in minor shell features and internal morphological traits such as radula teeth shape, number of ctenidial filaments, and characteristics of male and female sex organs.

Six widely dispersed species constitute the core of the Tamar clade in most analyses; two others, the Castra (Wilmot) species, *B. wilmotensis* and *B. phasianella*, are also associated with the group in the 16S analysis, but nest with the Castra group in the CO1 analyses. Species within the core Tamar clade express different morphological traits and are found in two PSGs while the inclusion of Castra (Wilmot) species introduces a third PSG (Table 6.3). Members of this group display a range of habitat requirements and a varied set of morphological characteristics. Five of the species inhabit small headwater tributaries, one occurs across a range of stream conditions from seepages to third order streams and two are riverine species occupying the underside of large, stable rocks. The riverine species (PSG 1) are of medium size (2.0 – 4.2 mm), rather globular (width/length ratio of 0.8 – 0.9) and have a simple penis. PSG 4c species are more conical to broad conic (width/length ratio 0.6 – 0.8), range in size (2.1 – 3.7 mm), have small to closed umbilicus and rounded to subangular periphery, while the single species in the third PSG (PSG 7) is smaller (1.9 – 2.3 mm), elongate (width/length ratio 0.6 – 0.7), with weak columellar fold and a simple penis (Ponder *et al.* 1993).

One possible explanation for much of the Tamar grouping is the formation of the Tamar River system, which was initiated in early Tertiary times by a period of faulting that resulted in the formation of the extensive lowland area (Burrett and Martin 1989). However, this does not satisfactorily explain the relationship with *B. hermansi*, *B. waterhouseae* and *B. forthensis* in this clade, a sub-group of species which occur in close geographical proximity to each other and which are more closely geographically associated to the Castra group, occurring in the lower regions of the Forth-Wilmot river catchment.

While uncertainty about the monophyly of *Beddomeia* remains, the separation of *Beddomeia* into consistent clades is well supported. Patterns of internal clade groupings only weakly support the phenetic species-groups concept detailed in Ponder *et al.* (1993). This groups species based on morphological similarities and was not intended to imply species relationships, but rather to reflect a strong pattern of spatial distribution, particularly evident in the separation of the NE and NW species (Figure 6.7), with geographically close species showing fewer base pair changes, indicating more recent speciation events. The phenetic species-groups identified by Ponder *et al.* (1993) were formed by clustering morphologically similar species together, without considering spatial relationships amongst the species, for example, the PSG 2 (*B.*

tasmanica group) combines species from across the NE and NW. Despite this, several of the phenetic species groups do contain similar groupings of species that also reflect spatial distributions (Figures 6.6 and 6.7); therefore, it is impossible to completely disassociate PS-groupings and distribution in explaining the phylogeny.

Strong biogeographical patterns have been observed in other aquatic and terrestrial invertebrate genera in Tasmania (e.g. Swain *et al.* 1982, Horwitz 1990, Eberhard *et al.* 1991, Ponder *et al.* 1993, Mesibov 1994, 1996b, 2000, Bonham 2003, Clark *et al.* 2003), aligning with phenomena such as Tyler's line, or geological historic events such as glaciations and faulting (Burrett and Martin 1989, Hansen and Richardson 2002). The phylogeny presented here adds further support to the invertebrate bioregions and faunal breaks proposed by Mesibov (1996b), particularly the Plomley's island and the East Tamar division, since all analyses showed a separation of the northeastern *Beddomeia*, but also the general divisions of the northwestern clades. However, due to the lack of *Beddomeia* specimens obtained from the far west of Tasmania in these analyses, full exploration of whether *Beddomeia* or *Phrantela* spp. recognise Tyler's Line as a faunal break is currently not possible.

Further exploration of geological history of Tasmania's north might also assist in explaining the distribution of some species and therefore the *Beddomeia* clades identified in the phylogeny. In recent geological history Tasmania has undergone at least three separate glaciation events and been subjected to frequent sea level changes (Burrett and Martin 1989). Through this process the highlands of northeast Tasmania have become isolated from the remainder of the State's uplands by the creation of the greater Tamar River catchment, extending from the Forth River valley in the central west to the Sideling range in the east, and draining the central plains from as far south as St Patricks Pass near Oatlands. This may, in part, be sufficient explanation for the molecular distinction of the NE and NW clades, although the monophyly of the genus by inference indicates a previous common, or multiple set of dispersal events. Also, the distributions of *Beddomeia* and *Phrantela*, in particular, coincide with regions of higher rainfall. Most *Phrantela* occupy habitat in the wetter west, southwest and northwest, while *Beddomeia* species occupy streams across the northern third of Tasmania's mainland, the majority of which are restricted to streams in mountainous regions. *Beddomeia* spp. display a broadly similar distribution pattern to *Astacopsis gouldi* (the giant freshwater crayfish) which frequents north-flowing rivers and streams across northern Tasmania, excluding the greater Tamar catchment (Swain *et al.* 1982, TSS 2006). Patterns of *Beddomeia* and *Phrantela* distribution also broadly reflect the distribution of burrowing crayfish of the genera *Engaeus*, (across northern Tasmania), *Ombrastacoides* and *Spinastacoides* (across northwest, western and southern

Tasmania) and it has been argued that historical processes determined the distributions of parasticid crayfish in Tasmania (Horwitz 1988, 1990, Hansen and Richardson 2006) and is therefore likely to at least partially explain the distribution of *Beddomeia*. *Beddomeia* however, for example those in the lower Tamar catchment, also occupy the larger river systems, from which *A. gouldi* is absent, and an unsubstantiated historic record suggests that *Beddomeia* may have extended as far south as St Peter's Pass near Oatlands, within the greater Tamar catchment (Australian Museum records).

Movement of snails between catchments is limited by their ability to disperse. While the clades show strong spatial relationships, several of the species within individual clades occupy adjacent catchments, which may be separated by less than 2 km, or on the opposite sides of ridges. How then might the phylogenetic relationships be explained? Uplifting at geological faults and river capture (also called stream piracy) incorporating previously unassociated streams into new catchments, have been documented in the geological history of Tasmania, New Zealand and in the U.S.A. (e.g. Waters *et al.* 2001, A. Richardson per comm., Hershler *et al.* 2008) and may at least partially explain movement of species between some catchments. Given the current knowledge of habitat requirements and life-history parameters of the *Beddomeia* species and the absence of suitable vectors (e.g. waterfowl) from forested catchments, it is unlikely that assisted dispersal has been a mechanism available to the *Beddomeia* species, adding weight to the probable importance of geological processes.

6.6 Conclusions

The monophyly of *Beddomeia* remains unresolved owing to the disparity of topologies retrieved from the analyses. The nesting of *Phrantela daveyensis tristis* in one *Beddomeia* clade, and likewise one *Austropyrgus* species, apparently renders *Beddomeia* paraphyletic, however, this result was not supported by all analyses and further work is required to resolve this relationship. Five *Beddomeia* clades were strongly supported by the analyses, reflecting spatial associations between species. However, speciation within certain clades was poorly resolved, with the 16S data suggesting limited molecular support for the specific status of *Beddomeia ronaldi* and *B. pallida* and questions the relationship between *B. briansmithi*, *B.* cf *minima* and *B. minima*, although the more conservative partial 16S gene sequence provides molecular support for the remaining named *Beddomeia* species in the analyses.

Bayesian clade credibility values for clades containing the morphotypes were 100%, however, there was less support for some individual morphotypes. Molecular support was revealed for the species status of some morphotypes including morphotypes C, 4 and 5, while the relationship between others remains unresolved due to a combination of insufficient genetic differences, unavailability of some sequences or suspect sequences. Two morphotypes were revealed to be closely related to previously described species, but the exact nature of the relationship is not clear as sufficient anatomical differences exist to question the relationships identified (e.g. between morphotype A, *B. wilmotensis* and *B. inflata*, and morph B with *B. hallae*).

This systematics study generally supports the taxonomy previously determined by Ponder *et al*. (1993). Sequencing of replicate specimens and *Beddomeia* species not included in this study will further elucidate this finding.

Section C Synthesis

This section comprises one chapter. It synthesises the information collected to address the main aims of the thesis presented in the preceding sections. In particular it reviews the information collected on the ecology and distribution of the species and suggests a conservation management approach for maintaining hydrobiid populations within forested landscapes.

7 Thesis synthesis

The fundamental aim of this thesis was to improve the biological and ecological understanding of the habitat requirements of *Beddomeia* species within production forests and to use this information to assist in the improvement of conservation outcomes. This research has addressed this goal by gathering information on the genetic relationships within the genus *Beddomeia*, by revealing ecological information on distribution, variability and population structures of *Beddomeia*, and through exploring the effects of disturbance. A particular strength of this research is the considerable effort devoted to the field collection and extensive dataset obtained.

The first part of this chapter summarises the main findings of this research, including a review of the survey methodology and the ecological data obtained. Recognizing these species as narrow-range endemics, the chapter reviews the requirements of narrow-range endemics and links this to the conservation requirements of threatened species and current mechanisms of habitat protection of aquatic species in the production forest landscape. It concludes with a brief discussion on how the information gathered in this thesis may be applied to the conservation management of these narrow-range endemics in Tasmania.

7.1 Ecology of *Beddomeia*

Distribution

The ecological requirements of narrow-range endemic invertebrate species like *Beddomeia* are seldom well understood, due in part to their diminutive size, difficulty in identification and limited appeal to many researchers.

Distribution of species throughout their ranges is infrequently homogeneous and is likely to be dependent not only on the availability of some key ecological factors and the absence of others, but also on the long-term historical continuity of these factors. Even for aquatic species existing in freshwater catchments, distribution and abundance may be variable across the landscape; such a result was demonstrated for the *Beddomeia* spp. in chapters 3 and 4. While this is important, it is not the complete story, and a strict ecological interpretation of current distributions that ignores the historical component will only be part of the story, a continuous history of suitable habitat over long periods of time is likely to be most important to understanding the current distribution of *Beddomeia*. Such information would greatly contribute

to the information of habitat requirements and ecological patterns affecting species, as insufficient or inappropriate knowledge may lead to ineffective conservation outcomes.

The scale of observation is an important consideration for identification of important habitat requirements and the results of this research clearly indicate a complexity in *Beddomeia* distribution that had not previously been considered. For example, in a number of cases, variability in *Beddomeia* abundance at the sub-site level was as high as, or higher than between certain sites and different streams of the same order (Chapter 3). An elevated level of narrow-range endemism is displayed by members of the genus and many species are known from only one site or small catchments, based on a broad, but not intensive study (Ponder *et al.* 1993), therefore it could be expected that *Beddomeia* are either present or absent from a stream. However, the finding of wider distributions and significant differences in abundance of *Beddomeia* species occurred between adjacent or localised streams, despite these streams displaying similar physical attributes and occurring in the same catchment, was unanticipated.

The scale of the study in Chapter 3 contributed greatly to our understanding of the distribution of *Beddomeia* spp. throughout large catchments and provided insight into the influence of geology on their presence, something previously not considered. Headwater streams and seepages were recognised as containing the greatest abundance of snails, although significant variation in numbers did exist. In hindsight, given their diminutive size, limited reproductive capacity and habitat (CPOM and rocks) choices, it may not be surprising that *Beddomeia* in this study exhibited a strong association with smaller, headwater streams and that snail abundance declined exponentially with increase in stream order; however, until this study was conducted, such catchment scale distributional data was unavailable for *Beddomeia*, or indeed other species of hydrobiids.

Micro-habitat preferences

The ecological requirements of the *Beddomeia* spp. studied were characterised by preferences for sites of low flow, small catchment size and availability and composition of micro-habitat material (allochthonous material (CPOM) and rocks), habitat requirements previously identified by Ponder (1997b). Despite proportionally higher recovery of snails from CPOM samples, interaction between CPOM and rock substrate confounded the identification of habitat preference, and owing to the sampling methodology, direct comparison of snail densities and total abundances was statistically invalid, due to differing surface area ratios.

Beddomeia spp. displayed a homogenous distribution on the underside of submerged rocks

raised off the substrate, but more clustered patterns on embedded rocks, the snails more frequently encountered at the water interface of rocks and substrate. Observations of the preference of *Beddomeia* for the underside of habitat material such as woody debris and rocks supports the behavioural traits recognised by Ponder *et al.* (1993).

Geological influences

Geological influences were identified as an important contributing factor to the distribution of *Beddomeia* spp. (and *Austropyrgus* spp.) in the Castra catchment and this raises the question of whether other *Beddomeia* and *Austropyrgus* species would respond differently to changes in geology. Although the catchments in this study were selected on the basis of different underlying geology (basalt vs granite), the ecological response to geological transitions within catchments was not a particular aim of this research. Insights identified here reveal an important gap in our understanding of the behaviour of *Beddomeia* spp. which may be crucial to improving management outcomes for these species at the catchment level; therefore it is proposed that future studies should investigate this phenomenon.

Response to disturbance and threats

Agriculture, forestry, mining and dam construction have previously been thought to have considerable negative impacts on aquatic mollusca (Ponder and Colgan 2002, Ponder and Walker 2003, Strong *et al.* 2008), in particular, *Beddomeia* populations (Ponder *et al.* 1993). Ponder (1997b) recognised at least three suspected extinctions in hydrobiids, including one *Beddomeia* species, *B. tumida* from Great Lake, and one undescribed species of *Phrantela* from the Serpentine River, since record keeping began in the 1800s, flooding and land clearing suspected as the likely causes. However, the limited ecological response to anthropogenic disturbance (forestry, mining) reported in this thesis indicates a level of tolerance of at least some *Beddomeia* spp. that previously was considered to be unlikely. Indeed, the recent re-discovery of *B. tumida* in Great Lake (Brad Smith, Hydro Consulting, pers. comm.) during record low volumes in 2008, indicates that this may also be the case for lake-inhabiting species. How other *Beddomeia* species respond may of course be different, and the possibility that extinction of undocumented species may have taken place in the Groom River catchment as a result of mining activities in the late 1800s - early 1900s cannot be discounted, although no evidence for this hypothesis exists, probably because of a lack of systematic and well documented early collecting (Ponder 1997b).

While cable-harvesting was shown in this study to have minimal impact on the *Beddomeia* population, the presence of a high density population upstream of the operation is likely to have

contributed to the survival of the population. Dispersal of snails downstream may occur through transportation on woody material during high flow periods, recolonising the disturbed stream reach and thus reducing overall observable impacts. Alluvial tin mining practices in the Groom River catchment caused severe channel erosion and modified channel features; such practices are likely to have caused local extinctions of hydrobiids in a number of headwater streams. Despite this, *Beddomeia* populations were found in 13 of the 15 small streams in this catchment; some populations occurring in large numbers. Although dispersal of snails (and other species, including fish), between streams via water races created during alluvial mining operations is possible (Waters *et al.* 2001), the recovery of snail populations after such disturbance suggests that these species of *Beddomeia*, at least, may be able to tolerate high-level disturbances, although the local extinction of genetically distinct populations cannot be discounted. Such evidence implies that *Beddomeia* spp. are capable of recovering from intense disturbance providing sufficient time exists between disturbance events for recovery to occur. Support for this hypothesis is offered by the findings of the cable-harvest investigation, observing that charcoal resulting from high intensity burning is not a hindrance to periphyton establishment and *Beddomeia* colonisation. However, this is not the case for land clearing, in situations where no regulation for riparian vegetation management exists. Supplementary evidence from sites of permanent land, such as for agriculture and rural-residential subdivisions, clearing suggest that long-term removal of riparian vegetation is detrimental to the native, stream-inhabiting, hydrobiid fauna, especially *Beddomeia*, where after >5 years local populations have been observed to decline significantly, and in one situation appear to have become locally extinct (K. Richards unpublished data). Such conditions also provide increased opportunities for the introduction of the exotic *Potamopyrgus antipodarum.*

Competition exerted by introduced species such as *P. antipodarum* may also be classified as a threat to native aquatic molluscs (Ponder 1997b). Evidence collected by the author seems to suggest that *P. antipodarum* has a preference for disturbed sites and only infrequently disperses into pristine native forest streams. In such cases *P. antipodarum* has been observed to outcompete *Beddomeia*, and to a lesser extent, *Austropyrgus* species; however, the impacts of this introduction on the aquatic molluscan fauna remain unstudied in Tasmania.

Natural variation in the population abundance over time may act to mask the effects of disturbance on *Beddomeia*, and the longevity of *Beddomeia* spp. may also disguise the effects in the short to medium term. Only through careful study of the population structure can we detect where lengthy interruptions to breeding events have taken place, and then only where sufficiently high population densities occur. It may be that shell microchemistry offers a more

accurate means of predicting the timing of such disturbance, in which case it will be necessary to obtain accurate growth rate and longevity data to determine snail age.

Population structure

The population structure data obtained greatly contributes to our understanding of *Beddomeia* life-history and response to disturbance. Normal patterns of population structure in undisturbed streams reflect low fecundity rates and suggest longevity within *Beddomeia*, the latter supported by evidence suggesting a life span of greater than five years and possibly as much as seven years (K. Richards unpublished data). The typical population structure is characterised by high percentages of adult and sub-adult members, contributing up to 70% of the total population, while juveniles and S1 generations represent less than 10% of the population. Similar configurations were exhibited in each catchment and between species indicating life-history patterns are common among the *Beddomeia* species investigated.

Observations of low egg capsule abundance at sites containing high population densities implies low fecundity, and the slow maturation rates of egg capsules, recorded from eggs collected in the field hatching between one to three months after collection (oviposition dates unknown), provide further support for this theory (KR unpublished data).

Beddomeia egg capsules do not readily survive exposure to air in dry conditions. Even under moist conditions, such as those observed following high flow periods where debris and rocks have been displaced but canopy cover is intact, viability of exposed eggs is reduced after several hours (KR personal observation). However, where sufficient habitat allows for retention of mature individuals, longevity may be beneficial, allowing *Beddomeia* to persist until breeding conditions improve, providing the disturbance is short term.

Not surprisingly, patterns in the structure of *Beddomeia* populations alter dramatically following intense disturbance to habitat. Recovery of population structure following cable-harvesting was observed to take between three to five years for most sites, i.e. for the configuration to return to cohort proportions similar to those in undisturbed streams, if not population density. It is probable that smaller populations will respond in a similar way, although recovery time may extend where low population density of *Beddomeia* occur.

Beddomeia and speciation (molecular vs morphological taxonomy)

Phylogenetic advances have improved our understanding of genetic variation occurring within species and the results obtained in this thesis contribute such information for *Beddomeia* spp.

While the monophyly of *Beddomeia* remains unresolved owing to the disparity of topologies retrieved from the analyses, and the probability of at least one misidentified sequence, and speciation within certain clades was poorly resolved, the species status of most *Beddomeia* is supported by the 16S and CO1 gene sequences.

Beddomeia spp. have been shown to occur sympatrically in many streams, and a number of species have been recognised at some locations; similar relationships were observed in this study (Ponder *et al.* 1993, Chapter 3). As indicated by Ponder *et al.* (1993), the convergence in shell characters and the small differences observed between some species make these features unsuitable for field identification of species. In this study morphotypes were originally recognised based on shell characteristics; however, when shell characters are ignored, we see that insufficient morphological differences exist to support the number of morphotypes first identified, but that we can recognise at least two species from each catchment. DNA evidence supports the taxonomy derived from the anatomical comparisons conducted in Chapter 5, but also raises questions about the relationships between some morphotypes and named species, suggesting that further work is necessary to reliably establish the taxonomy.

7.2 Limitations and Improvements

Review of sampling method

Obtaining quantitative measurements of absolute population density is not possible using the sampling method documented in chapters 3 and 4. The method used was repeatable and effective, and was successful in replicating sampling effort between sites, and particularly suitable for establishing presence of *Beddomeia* in low abundance situations. However, further refinement of the methodology would be required to determine the nature of any specific habitat preference (e.g. rocks in preference to CPOM), specifically whether a habitat preference exists or whether opportunistic behaviour dictates snail presence.

The sampling methodology was unable to determine the depth to which *Beddomeia* utilise the streambed substrate; however, given the cryptic nature of the species, occupying the underside of rocks and CPOM, it is unlikely that *Beddomeia* would spend much time on the streambed surface. Some level of burial is more probable, but the depth to which they may occur remains unknown, although it is likely to be dependent on food availability. Evidence of *P. antipodarum* behaviour indicates this species at least is able to utilise substrate greater than 5 cm depth. A more appropriate method would need to be developed to determine whether the snails are

capable of movement through substrate and would need to consider the influence of geology, as it is likely that species may respond differently in substrates with large interstitial spaces compared with dense siltation, streambed compaction, or different geological types.

Population studies

Population studies can provide insightful results, but to achieve this, they require a sufficiently large number of snails to be statistically accurate and representative. The method developed was useful for determining population structure where snail abundance was high (> 50 snails per sample), but careful consideration is required in the design of a sampling regime to accurately reflect the population structure in low abundance populations, and to replicate sites and treatments so as to be able to generalise more confidently.

Prior to the study, the breeding behaviour of *Beddomeia* was unknown. The sampling design incorporated a seasonal element in an attempt to establish whether a breeding pattern exists. Given the variability in snail population abundance between sub-sites (representing seasonal patterns), sites and streams, it was not possible to fully test the existence of a seasonal cycle; however, the population structure data overwhelmingly indicate that no cycle exists, suggesting continual or frequent breeding. A similar pattern is likely to be the case for other *Beddomeia* spp., but would need to be confirmed. This suggests a further avenue of research, to determine the fecundity of individual *Beddomeia* species.

While there are some drawbacks with the sampling methods applied, they are useful in obtaining large quantities of snails of all age cohorts which allow population abundance data for *Beddomeia* spp. to be gathered. This level of detail of hydrobiid life-history has not previously been studied, and the results have revealed some interesting patterns of response to disturbance which should be further investigated. Benefits of the application of this method for future studies include the ability to obtain data from individual habitat types (rocks or CPOM, or specific types of CPOM), short field-collection time and ability to collect associated macroinvertebrates and to survey multiple aquatic mollusc species concurrently, to investigate patterns of spatial distributions.

Application of research

This study provides information that may help with the conservation management of the species investigated; however, extrapolation of the results of the current study to other *Beddomeia* species should not be attempted without first obtaining information on the species' spatial habitat requirements (e.g. preferred position within the catchment), but where similar, the

findings may benefit the species through adoption of similar conservation measures. Caution is necessary, especially in cases where geological transitions exist within the species' range, as the tolerance level of other *Beddomeia* species to geological shifts is unknown. There is also need for caution applying these findings to riverine and lake-inhabiting species. To date, we have limited understanding of the environmental requirements of these species, but it is likely they will respond to a different set of environmental stimuli.

Current knowledge suggests that the data obtained are specific to a subset of *Beddomeia* species and should not be extrapolated to other Hydrobiidae, or closely related families, in Tasmania without careful consideration of the consequences. For example, members of the genus *Austropyrgus* co-occur with *Beddomeia* in many instances, but not all, and are absent from the Groom River catchment despite occurring in nearby streams. The distribution of *Austropyrgus* spp. and *Beddomeia* spp. in the Castra catchment was noticeably different; *Austropyrgus* spp. were frequently observed in high numbers in areas where *Beddomeia* spp. were absent or in low abundance. Therefore, application of these findings may not benefit such species without further understanding of the requirements of *Austropyrgus* spp.

Consideration is also required before extrapolating from the findings of the cable-harvest study for a similar reason; it is impossible to know to what extent the population density influenced the observed response and it is likely that snails under reduced population pressures would respond differently. Results of a more intensive before and after controlled impact investigation (BACI) conducted in other areas, and on other species, are likely to provide missing data. Such a study is currently in progress for a related species, *Phrantela pupiformis*, in southern Tasmania (Davies *et al.* 2009) and will contribute valuable information. Further studies need not necessarily require such lengthy study periods, or be conducted for each snail species, rather, they could utilise the results of longevity studies and life-history data to assist in interpretation of population structure data taken within the first – third year following harvesting to interpret trends.

7.3 Conservation and management issues

Factors which characterise a species' geographic range and its "propensity for population differentiation and speciation" have previously been reported in the literature (e.g. Blackburn and Gaston 2001, Ponder and Colgan 2002): a combination of intrinsic and extrinsic factors influencing a species' geographic range (Chapter 1). Those species with specific habitat

requirements and which are poor dispersers tend to have smaller geographic ranges; many such species are considered narrow-range endemics, *Beddomeia* amongst them. The habitat requirements of many of these species are unknown, as are the consequences of altering habitat features within their range. For such species a precautionary approach is often applied to their management, based on predicted or potential threatening processes, in lieu of population size or life-history data, which are equally important considerations in the management decision-making process.

Legislation in place to protect narrow-range endemic species in Tasmania includes the Tasmanian *Threatened Species Protection Act*, 1995, *Nature Conservation Act, 2000*, and *Environment Protection and Biodiversity Conservation Act, 1999* (Chapter 1). Those species with ranges in production forests are managed through agreed biodiversity management processes that take a 3-tiered approach, with measures to protect species habitat at the local and landscape levels as well as specific management prescriptions for each species or group of species (Forest Practices Board 2000a, 2001). While the ranges of most *Beddomeia* species occupy sites within the forest estate, several other *Beddomeia* species (more endangered) occur in lowland river systems in areas not subject to forestry activities. In such cases these species receive little or no protection. Indeed, there is an almost complete lack of convergence between recognised *Beddomeia* spp. ranges and current protected areas (Ponder 1997a, 1997b).

From the results of this study we now know that the distributions of at least a few *Beddomeia* species are larger than previously anticipated; such information can be used to predict that the ranges of other species are also likely to be extended. This will be particularly useful in determining range extensions for species whose habitat requirements are known. Based on these findings, we can also predict with high likelihood, that more, as yet undescribed, species and subspecies of *Beddomeia* occur in Tasmania. If, indeed, the diversity of *Beddomeia* spp. is greater than is currently known, and they display similar attributes to species currently accepted, then many of these undescribed species are likely to fit the criteria for narrow-range endemism, and therefore, undoubtedly will qualify as „threatened' under the current threatened species legislation.

This study has substantially contributed to our understanding of the ecology and taxonomy of a molluscan group of conservation interest and has assisted in gaining information about the *Beddomeia* genus that can assist in management decisions, finding that: (1) there is DNA support for the current species-level taxonomy of *Beddomeia*, and that more species or subspecies exist, (2) some *Beddomeia* species are more widespread, albeit remaining narrow-

ranged, than previously known, (3) some *Beddomeia* spp. have higher than anticipated levels of tolerance to high level disturbances in the short to medium term, (4) *Beddomeia* spp. appear not to express strict preferences for habitat type, although support is given to the previous assertion that *Beddomeia* spp. show a preference for the underside of rocks and CPOM, (5) some *Beddomeia* spp. display aversion to particular geologies, and (6) *Beddomeia* spp. display low fecundity and are relatively long-lived. What then, are the implications of these research findings for the management of *Beddomeia* spp.?

Management of listed species of *Beddomeia* spp. in production forest is provided through a combination of landscape reservation, local informal reservation, stream management and specific prescriptions (Forest Practices Board 2000a, 2001). However, the value of landscape level reservations for the protection of *Beddomeia* spp. is limited; thus Ponder (1997a, 1997b) demonstrated the disparity between location of formal reserves and predicted *Beddomeia* spp. ranges. Until now the precautionary principle has been applied to *Beddomeia* spp. management. This approach dictates that where there is uncertainty or lack of available information concerning possible adverse effects on a species, management decisions should be made in favour of the species (Morrison-Saunders and Arts 2004). As such, a minimum 10 m intact streamside reserve prescribed for streams in catchments containing known populations of threatened hydrobiid snails, and that no more than 15% of a catchment containing a listed hydrobiid species should be harvested within a 10 year period (Forest Practices Board 2000a, 2001).

Management of *Beddomeia* spp. has to date been in the form of genus-level prescriptions delivered on a ‚presence in catchment' basis for each threatened species, but this approach if not applied accurately is costly (to the industry and landowners) and ineffective for species conservation. There remain issues with data accuracy, (conversion of datasets and inaccuracy of handheld GPS) putting some records at least 5 km away from where they were collected, and interpretation of the definitions of terms such as ‚catchment', ‚catchment containing' and ‚class 4 stream[1] (generally encompassing first and second order streams)', that have repercussions for species conservation outcomes. This is particularly true where misinterpretation of such terms may lead to incorrect determination of species absence, and is compounded by problems with identification of species, requiring specialist taxonomic skills to identify *Beddomeia* past genus level.

[1] Forest industry headwater stream classification (< 50 ha) (Forest Practices Board 2000)

To date, a review of the effectiveness of these measures has not been undertaken, but preliminary observations of on-ground practices suggest that some improvements to the system need to be made, at least for some species such as *Beddomeia fultoni, B. ronaldi*, *B. hermansi* and *B. wiseae* which have been surveyed and are known from only a handful of streams in rural and forested areas. (K. Richards personal observation, FPA unpublished data). The habitat for such species is under threat due to agricultural clearing and large scale conversion to *Eucalyptus nitens* plantations. Therefore, the fundamental question is what measures should be used to improve the management of threatened hydrobiid species: and should they be managed as individual species, for biodiversity values, at the catchment level, by phenetic species-groups, or a combination of approaches? Results of the current research raise a pertinent question, given the findings of this research is whether a call for improvements to management prescriptions is necessary? And raises further questions of how many *Beddomeia* species need to be studied to determine whether the responses observed here apply to other species within the genus; and, without such data, should we continue to apply precautionary management of these species?

By way of addressing these questions, a number of management options have been put forward:

Option 1: Species specific management approach

Single species management is time consuming, requiring multi-agency input and in-depth knowledge of the species of concern. Careful mapping of range boundaries for narrow-range endemics is not cost-effective (Mesibov *et al.* 2002), even before the costs associated with gathering life-history data are included. Management of single species becomes ineffective when knowledge is lacking, as is the case for many invertebrates, and so a precautionary approach is often adopted, but can be over-prescriptive where limited information exists (Morrison-Saunders and Arts 2004).

Information gained from the small number of *Beddomeia* species studied can assist in the development of management prescriptions that will benefit others; however, as indicated above, the findings obtained here may not be reproduced for other species, and therefore a degree of precaution is still necessary. One approach to the individual species management may be to divide *Beddomeia* spp. into groups based on the information available, i.e. data rich (such as for these species), versus data poor (such as where only range boundaries are known), or those for which no information is available, and to manage them accordingly. Such groupings are likely to be based on habitat preferences; for example headwater vs large stream species. As this research is the first of its kind for *Beddomeia*, the first group is likely to be small, the second will contain about 16 species with the majority of species making up the final group.

Application of the precautionary principal would, by necessity, apply to data-poor species, but unlikely to apply to riverine species as the known sites of many are outside of forested areas, thus protection measures do not apply. Therefore, such an approach is unlikely to vastly improve the current approach.

Option 2: Multiple-species management approach

It is frequently assumed that many species of freshwater invertebrates have similar habitat requirements, be they for streams or lakes, headwaters or larger catchments. A second approach (option 2) could be to manage the group as a whole through habitat management using intact stream buffers, with the assumption that species protection will follow; however, this might not be an appropriate approach to use for *Beddomeia* spp, or other narrow range endemics, as highlighted in Ponder (1997b).

Option 3: Landscape level management approach

A third approach (option 3) could be to manage *Beddomeia* at a landscape scale, through reservation of a proportion of any given catchment, managing the remaining habitat with little or no regard for the species. This approach forfeits management of a significant component of catchment in the short-term, but is a measure proposed to protect levels of in-stream CPOM by Watson (2004) and is also an option put forward to protect stream biodiversity in a recent review of the forest practices code (Barmuta 2009).

Results from this study suggest that the original assumption (that *Beddomeia* spp. have similar requirements and can be managed as a group) is incorrect, recognising that individual *Beddomeia* spp. do have unique habitat requirements, and finding that not all streams are equally important to individual species. Therefore, option 1 has some merit, increasing precautionary management for species for which we have no, or limited, data, but based on current information, the majority of species would remain in group 3.

Option 2 is similar to the current situation for management of *Beddomeia*, but without the additional restriction limiting harvesting to a percentage of the catchment (for most species). Management prescriptions designed to cater for the group as a whole must either be over-prescriptive (precautionary) or else likely to be ineffective for some species. However, provided that an agreed riparian reservation width is established, and that measures are in place to minimise water quality impact and to prevent burning of riparian vegetation, this approach would seem to be an acceptable management outcome for at least some *Beddomeia* species.

This approach is somewhat limited by the paucity of information on the disturbance tolerance of *Beddomeia* spp. and it may be that some species are more sensitive to disturbance.

The third option (option 3) would only be effective with prior knowledge of the range of species across the catchment landscape, including abundance data, which are rarely available. This approach assumes that streams will be equally important to all species, a fact shown to be incorrect for the *Beddomeia* spp. investigated in this study, and therefore is unlikely to be an effective conservation mechanism, for this group at least.

Management by phenetic species-group or indicator species are also options worthy of consideration, as is the use of population viability analysis as a tool to determine the most appropriate management decisions.

Option 4: Phenetic species-groups management approach

The classification of *Beddomeia* species into phenetic species-groupings was primarily based on similarity of morphological traits and never meant to imply species relationships (Ponder *et al.* 1993). The findings presented in Chapter 6 reveal that while there are some similarities in traits within the phenetic species-groups and groupings generally correspond to invertebrate faunal gaps and bioregions, sufficient differences exist between these groupings and molecular phylogenetic clades to mean that management using such groupings is inappropriate. Additionally, the habitat preferences of species in some phenetic species-groupings differ, ranging from headwater specialists to large stream inhabiting species, confirming that a management approach using these groupings is considered unsuitable.

Option 5: Indicator species management approach

Many waterway management authorities recognise „indicator species' as a useful means of assessing the health, or otherwise, of systems under their care, but rarely, if ever, is this approach applied using a single species, or headwater streams, most researchers preferring to use a rapid assessment approach, using numbers of Trichoptera, Plecoptera and Ephemeroptera to assess river health (e.g. Davies *et al.* 2005a, Smith *et al.* 2009). It has been suggested that aquatic molluscs might be useful as indicator species and would benefit from a raised level of awareness and public image (Ponder 1994, Seddon 1998, Strong *et al.* 2008). Their size and often large numbers make them practical tools for bioassessment, but often these are usually marine, or common species (Lee *et al.* 2002). Indicator species are usually species that show some level of sensitivity to pollutants or other changes to a system and the potential to use freshwater aquatic molluscs as indicators has been recognised (Strong *et al.* 2008). However, it

remains to be seen whether narrow-range endemics should be used for this purpose. While *Beddomeia* spp. may serve as a useful umbrella species (Romberge and Angelstam 2002), such a option would require each stream in every forestry operation to be surveyed, something that is unrealistic in the current financial climate. Although the practical implications of such an approach generally applied are daunting, this is however, seen as an appropriate conservation management approach for areas where *Beddomeia* is known to occur, or adjacent to those areas.

Option 6: Population viability analysis (PVA)

PVA might further assist with future management decision s for *Beddomeia* species. Using the data obtained from this study as a template, it may be possible to construct a crude model to measure the impacts on this group and to identify which conservation measures are most appropriate. In order to undertake such analyses, a series of tasks first need to be undertaken; and include: collating existing data, costing and ranking of management options (Possingham et al. 2001). The benefits of PVA are in providing managing authorities with insight into the predicted effects of management decisions (Lindenmayer and Burgman 2005). However, PVA works best at the species level, and without supplementary information from other *Beddomeia* species, such analyses are likely to benefit only a subgroup of *Beddomeia* species.

7.4 Recommendations

While narrow-range endemicity and low fecundity are attributes of *Beddomeia* suggesting a precautionary approach to species management be applied, the resilience of the *Beddomeia* spp. to disturbances such as mining and forestry identified in this study is encouraging and suggests that a review of the management protocols for *Beddomeia* is needed. Despite being narrow-range endemics, the *Beddomeia* spp. investigated here have demonstrated tolerance of high-level disturbance events. To date such resilience has previously been considered unlikely (Ponder 1997b), and the precautionary principal has therefore been applied to their management in areas subject to forestry activity. Extrapolating from the results of this research is problematic as, while it is likely that other species of *Beddomeia* will display similar responses to disturbance, inevitably this will not be the case for all species. Indeed, Ponder *et al*. (1993) collected some species of *Beddomeia* from streams of less than pristine conditions (K. Richards personal observation), and while this is encouraging, "probable" extinctions of hydrobiids have been reported (Ponder 1997b). One of these likely extinctions, *Beddomeia tumida*, resulting from the permanent habitat inundation from the flooding of Great Lake, in central Tasmania, has recently been rediscovered (Brad Smith, Hydro Consulting, pers. comm..). Some

disturbance events such as agricultural clearing leaving no riparian buffer strips, and stream diversions, are too extreme, causing local population extinctions (K. Richards personal observation) and the importance of riparian zones to the conservation of fish and macroinvertebrates have been documented (e.g. Everett and Ruiz 1993, Sheldon and Walker 1998, Crook and Robertson 1999, Pusey and Arthington 2003), but little has been reported on freshwater mollusc requirements (Ponder 1997b). Therefore, it would be unwise to reduce the level of management protection for all species, ‚putting all the eggs in one basket', until further research is conducted to determine whether similar responses to high-level disturbance are observed in lower abundance snail populations. Meanwhile, there are approaches to management that can utilise the results of this research to the benefit both industry and *Beddomeia* spp.

It is proposed that a combination of management approaches be considered for *Beddomeia*. First, for ‚data rich ‚species such as *B. hallae*, *B. wilmotensis* and *B. tasmanica*, application of option 3, landscape level management approach, is seen as the most appropriate for this group in most situations. This may require lessening of the 10 m intact buffers on some headwater streams within the range of the species', but would afford protection to clusters of headwater streams known to contain particularly high densities of snails in situations where high percentages of catchments have already been modified (e.g. within the ranges of *B. hallae* and *B. wilmotensis*). Where high-level disturbance events are proposed, such as cable-harvesting (incorporating stream headwaters), mining, or agricultural clearing, these may still need to be addressed on a case-by-case basis, depending on the species concerned.

For the remaining ‚data poor' species, a combination of option 2 and 3 is required to ensure that, (1) habitat is protected, and (2) species are considered at the landscape level. This may require additional surveys to identify range extensions or high density populations. For these species, all high-level disturbance events proposed, within any ‚known range' will need to be addressed on a case-by-case basis. As less information is available for these species, a minimum 10 m intact streamside buffer should continue to be enforced on streams known to contain threatened species.

As a matter of urgency to advance management outcomes, improvements need to be made to database accuracy, and education forest industry personnel should be addressed to ensure the intent of the management prescriptions is not lost. A review of the landscape-level management tools should also be undertaken. While this study has shown some *Beddomeia* species are able to tolerate high levels of disturbance, additional pressures of climate change have not been

considered, and to date, remain untestable. To ensure long-term persistence of all *Beddomeia* species, a good beginning would be to improve reservation at the landscape scale, incorporating the habitat of narrow-range threatened species into new reserves. Serious consideration should also be given to increasing the streamside reserve protection on *all* headwater streams rather than only for streams known to contain a threatened species, and enforcing protection against damage from operational burns, thus allowing for a level of stream recovery to enter the system.

7.5 Future research

This study identified significant variability in snail abundance between streams, and the consequences of this finding need to be addressed by land managers prior to development of a detailed conservation management plan. The development of any plan should consider the cost of opting for retaining a proportion of catchment (potentially missing high density snail populations) vs improving riparian management overall, as the outcomes are likely to be very different. Consequently, prior to moving toward development of a genus-level management plan, this study has identified further avenues of research that must be addressed to improve our understanding of this molluscan group of considerable conservation interest.

Identifying the response of low-density populations to high intensity disturbances is recognised as a priority. Such a study would need to consider variation in snail abundance between sites and should, if possible, be of a BACI design to establish baseline data trends prior to harvest.

Excepting *B. launcestonensis*, riverine *Beddomeia* species have been paid little attention by land managers and researchers. It is likely that these species respond to a different set of environmental stimuli to those required by the headwater stream species in this study, but research is required to determine what these are. Additionally, the habitat preferences of such species need to be investigated and their ranges mapped. The recent re-discovery of the only recorded lake-inhabiting species (*B. tumida*) is promising, however, nothing is known of this species habitat requirements and research is necessary to gain a better understanding of the ecology of this species.

While this study has gathered intriguing preliminary life-history data of *Beddomeia* spp., further effort is needed to obtain more precise life-history data for the genus, as well as to identify any differences between headwater and riverine species. Such data will assist identifying effects of disturbances, particularly if shell microchemistry can be used to more accurately predict the

timing of such disturbance.

Finally, although the molecular investigation has laid the groundwork for an understanding of the taxonomy of *Beddomeia* species, further work is required to complete the phylogeny for all species. The study also uncovered some intriguing questions about the relationship between *Beddomeia*, *Phrantela* and *Austropyrgus* that need to be addressed.

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